



Case review

Post-mortem interval estimation based on insect evidence in a quasi-indoor habitat

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ABSTRACT

Insects collected on indoor cadavers are frequently used for post-mortem interval (PMI) estimation. Buildings encountered during crime investigations vary according to temperatures inside, the extent of insect access restriction or sanitary conditions. This article reports the PMI oriented analyses of insect evidence sampled from the human cadaver in the atypical indoor habitat. The body was found in the uninhabited house, on the floor covered with rubbish, in the room with no doors and windows. Thermal conditions in the room were less variable than in the local weather station, however still much more variable compared to the typical indoor habitat, indicating the need for retrospective correction of temperature records from the station. Cadaver entomofauna was surprisingly diverse and abundant. We recorded several taxa usually not occurring on indoor cadavers, e.g. immature stages of *Necrodes littoralis* (Coleoptera: Silphidae) or *Stearibia nigriceps* (Diptera: Piophilidae). PMI was based on the age and the pre-appearance interval estimated for live puparium of *S. nigriceps*, giving the total interval of 37 (± 7.4) days plus 4–20 days resulting from the absence of first colonizing specimens of the species. This estimate was corroborated with the age estimate for empty puparia of *Sarcophaga argyrostoma* (Diptera: Sarcophagidae) with traces of *Nasonia* sp. (Hymenoptera: Pteromalidae) eclosion. Other insects indicated shorter but consistent PMI. Difficulties and limitations of insect-based PMI estimations in unusual indoor habitats are discussed.

1. Introduction

Human cadavers are frequently discovered in indoor habitats. In the large-scale reviews of forensic cases with insect evidence, about 40% of cases comprised human bodies in indoor settings [1–4], although in some areas it was almost 80% [5]. When comparing indoor and outdoor habitats, cadavers inside dwellings are found to be colonized by insects with a delay of up to several days, depending on the extent of insect access restriction, temperature inside and the efficiency of attractants diffusion [6–9]. Experimental and case studies have regularly indicated that carrion entomofauna is distinctly less abundant and diverse on indoor cadavers [1, 6–11]. Accordingly, decomposition inside buildings is slower, resulting in prolonged insect colonization phase [8]. Another distinctive feature of indoor habitats are steady thermal conditions. Although buildings vary according to inside temperature, they have much smaller daily temperature variation than outdoor habitats [6, 12].

Some peculiarities of indoor cadaver decomposition and colonization by insects facilitate estimation of post-mortem interval (PMI). Stable environment inside buildings simplify the reconstruction of

temperature conditions. Moreover, most data on development of forensically important insects were collected at constant temperatures [13–16], therefore their longitudinal thermal profiles better correspond with thermal profiles of indoor than outdoor habitats. Some inherent difficulties of PMI estimation are connected with indoor settings as well. The extent of delay in cadaver colonization by blow flies or flesh flies is difficult to predict. Temperature methods for the pre-appearance interval (PAI) are not useful for the estimation of blow fly or flesh fly PAI [17, 18]. Besides, colonization times of indoor cadavers by blow flies or flesh flies have been poorly documented experimentally. Further, low abundance and diversity of entomofauna on indoor cadavers, and in particular low number of late-colonizing insects [1, 6, 9, 11] may hinder PMI estimation in long PMI cases. Finally, the elongation of insect colonization phase increases the chance that immature insects sampled will not be the first insects of the species that have colonized the cadaver, and this may enlarge the error of PMI estimate.

A neglected issue in forensic entomology is the variety of indoor habitats (but see [10, 12]). Buildings vary in entomologically important factors. The extent of insect access restriction is probably the most important. At one end there are buildings which do not limit insect

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access in any way, e.g. ruined houses with no windows or roof, and at the other end there are structures to which insects have no access, e.g. tightly sealed rooms. In forensic entomology at least two scenarios are typical: abandoned and unsecured buildings in which insect access is usually only slightly limited and inhabited or secured buildings in which restrictions are moderate or large. Temperature conditions inside buildings may also vary. At one end there are basements in which steady and rather low temperature usually limits and sometimes prevents insect activity [12]. At the other end there are attics in which thermal conditions are much more variable across the year. Indoor habitats differ also according to sanitary conditions inside [10]. At one end there are sterile houses in which insects that had colonized the cadaver must have arrived from the outside, at the other end there are dwellings in poor hygienic conditions where saprophagous insects are permanently present.

Here, we present a case report in which PMI was estimated based on insect evidence from a quasi-indoor habitat.

2. Case report

2.1. Case circumstances

The body of a no name adult male was discovered on August 6th, 2017 in the suburbs of Poznań (Western Poland). The body was laying on the floor, next to the window in the ground floor room of the detached house. The head of the cadaver was in the remains stage, torso and limbs were partly in the advanced decay stage and partly in the remains stage sensu Payne [19]. The pathologist estimated PMI at minimum three months. Due to the advanced decomposition, the cause of death has not been determined. There were no signs of third party participation. Documents from the hospital confirming the terminal condition of the deceased were found next to the body. The latest document was issued on May 26, the day when the man was probably last seen alive. The house was uninhabited, although there were traces of frequent presence of local drunkards and homeless people. At the ground floor doors were absent and all windows were broken (Fig. 1). Ivy grew into the house through the windows, indicating the long-term absence of windows (Fig. 1). In the room where the body has been found, the floor was covered with bottles, old clothes, food waste and other rubbish (Fig. 1).

2.2. Cadaver entomofauna

Insects were sampled by law enforcement officers during the cadaver examination in the building and by authors of the article during the second inspection of the building, during the pre-autopsy examination of the body and during the autopsy.

Cadaver entomofauna was surprisingly diverse and abundant (Tables 1–2, Fig. 2). Among Diptera there were larvae and puparia of various blow flies, large number of puparia of flesh flies with and without traces of *Nasonia* sp. eclosion, a few immature specimens of Muscidae and Fanniidae, many third instar larvae and a few puparia of Piophilidae. Among Coleoptera four species of *Dermestes* were sampled in the adult or the larval stage. Surprisingly, we found a few exuvia of second instar larvae of *Necrodes littoralis*. Moreover, several species and life stages of *Necrobia* were present on the body. Among Hymenoptera we found many adult *Nasonia vitripennis* associated with the body.

2.3. Temperature conditions

Temperature was recorded in the building from August 10th until August 17th with HOBO U23 Pro v2 2x External Temperature Data Loggers (Onset Computer Corporation, MA, USA). Conditions in the room were compared against temperature records from the local weather station using linear regression. The resultant regression equation was used to retrospectively correct temperature records from the



Fig. 1. The front side (a), the hind side (b) of the building and the general appearance of the room (c), where the body has been found.

weather station.

Temperature conditions in the room were less variable than in the weather station (Fig. 3; average daily amplitude, room: 4.0 °C, station: 8.3 °C; weekly amplitude, room: 8.6 °C, station: 14.8 °C). However, they were still distinctly more variable than in a typical indoor habitat (for comparison see data from Michalski, Nadolski [12]). There was a strong correlation between temperature in the room and in the weather station (linear regression, Room temperatures = 9.6873 + 0.5364 * Station temperatures, $r^2 = 0.89$, Fig. 3). Accordingly, we used the regression equation to retrospectively correct temperatures from the station to be used in PMI estimation.

Table 1
The list of Diptera recorded on the body or in the cadaver discovery place.

Family	Genus/species	Stage	Specimens recorded/sampled				Rearing
			6/7 VIII ^a	8 VIII ^b	8 VIII ^c	11 VIII ^d	
Calliphoridae	<i>Protophormia terraenovae</i> R.-D., 1830	L3				2	
		EP	1	1			
		I				1	
Sarcophagidae	<i>Phormia regina</i> Meig., 1826	L3		2			
		L3		1			
	sp.	EP		5			
		<i>Sarcophaga argyrostoma</i> R.-D., 1830	EP	2			
		<i>Sarcophaga</i> sp.	EP/ <i>Nasonia</i>	2			
Muscidae	<i>Hydrotaea</i> sp.	EP	Many	Many	Many	Many	
		P/dead		3		4	
		EP/ <i>Nasonia</i>		Many	Many	Many	
		L3				1	
		L2				1	
Fanniidae	sp.	L3		3	1		
		L3				1	
		L3				4	
Piophilidae	<i>Stearibia nigriceps</i> Meig., 1826	P		1			I/13.VIII
		L3	Many	Many		Many	
		P	1				
		EP		1		3	

L2 – second instar larva; L3 – third instar larva; P – puparium with alive pupa/pharate adult inside; P/dead – puparium with dead pupa/adult inside; EP – empty puparium; EP/*Nasonia* – empty puparium with traces of *Nasonia* eclosion; I – imago.

^a Cadaver examination at the place of discovery by law enforcement officers.

^b Cadaver examination at the morgue by authors of this report.

^c Cadaver discovery place examination by authors of this report.

^d The autopsy.

Table 2
The list of Coleoptera, Hymenoptera and Lepidoptera recorded on the body or in the cadaver discovery place.

Order	Family	Genus/species	Stage	Specimens recorded/sampled				Rearing	
				6/7 VIII ^a	8 VIII ^b	8 VIII ^c	11 VIII ^d		
Coleoptera	Dermestidae	<i>Dermestes lardarius</i> L., 1758	L	1	7	2			
			I	1	2		1		
		<i>Dermestes undulatus</i> Brahm, 1790	L		2				
			I			2	1		
		<i>Dermestes murinus</i> L., 1758	L			1			
			I			2			
		<i>Dermestes haemorrhoidalis</i> Kuster, 1852	I			1			
		<i>Dermestes</i> sp.	L	Many	Many	Many	Many		
		Histeridae	<i>Saprinus semistriatus</i> Scriba, 1790	I			1		
				I	1 ^e				
	Silphidae	<i>Necrodes littoralis</i> L., 1758	I						
			E/L2				12		
	Cleridae	<i>Necrobia rufipes</i> DeGeer, 1775	L2			2			
			L3		2	2		I/9.IX I/11.IX	
			I		1		1		
L2					1				
<i>Necrobia ruficollis</i> Fabr., 1775		L3		4	1	2	I/7.IX		
		P				1	I/28.VIII		
		I			2				
		I		1		1			
Nitidulidae	<i>Nitidula carnaria</i> Schaller, 1783	L1, L2		7					
		P		1					
Hymenoptera	Pteromalidae	<i>Nasonia vitripennis</i> Walker, 1836	L3			4			
			I		Many				
Lepidoptera	Tineidae	sp.	L		1				
			P		2				

L – larva of the unspecified instar; L1 – first instar larva; L2 – second instar larva; L3 – third instar larva; E/L2 – exuviae of the second instar larva; P – Pupa; I – imago;

^a Cadaver examination at the place of discovery by law enforcement officers.

^b Cadaver examination at the morgue by authors of this report.

^c Cadaver discovery place examination by authors of this report.

^d The autopsy.

^e The specimen recorded only in the picture.

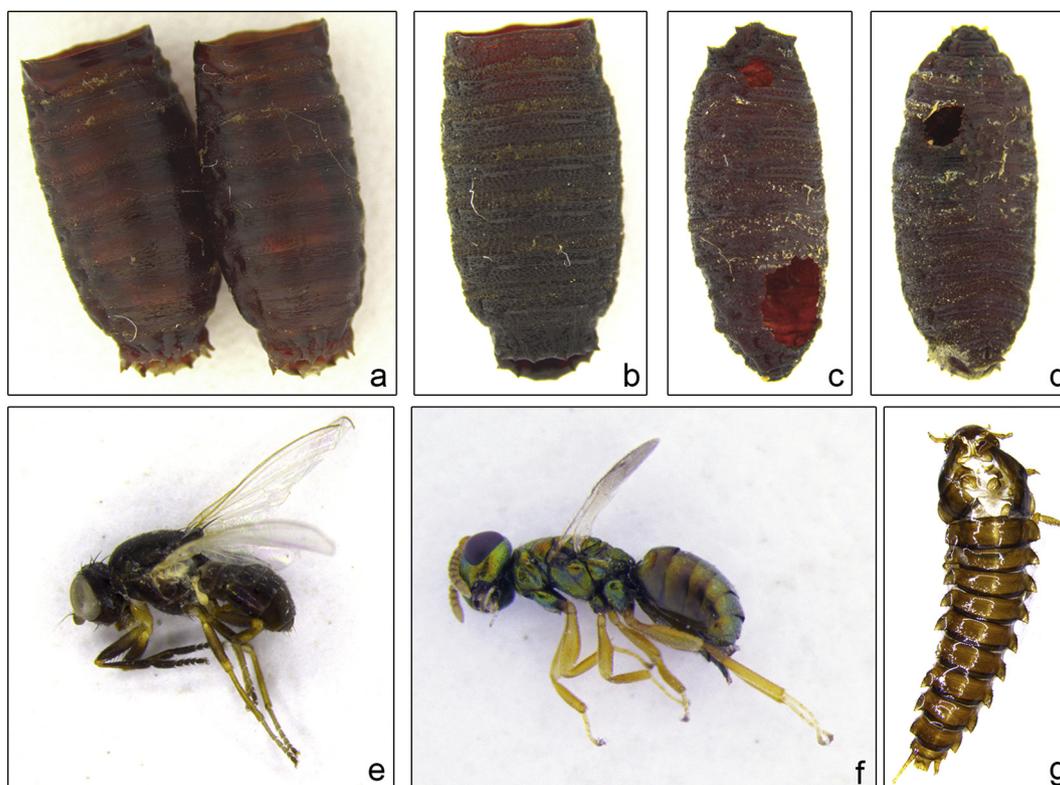


Fig. 2. Empty puparia of *Protophormia terraenovae* (a), empty puparium of *Sarcophaga argyrostoma* (b), puparia of *Sarcophaga argyrostoma* with traces of *Nasonia* eclosion (c,d), adult stage of *Stearibia nigriceps* (e) adult stage of *Nasonia vitripennis* (f), exuviae of the second instar larva of *Necrodes littoralis* (g).

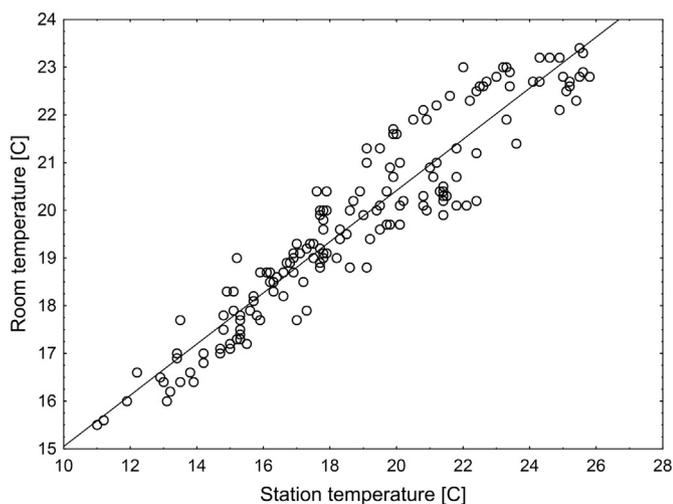


Fig. 3. The relationship between temperatures in the room where the body has been found and in the weather station.

2.4. PMI estimation

In general, insect age was estimated using thermal summation method [20–22], and supplemented with the PAI estimated using temperature methods [23, 24]. Because larval PAI may be estimated with the average error of about 20% [23] and because there is no contemporary data on the error rate of insect age estimates (it is probably lower than 20%), we decided to include a conservative 20% error rate for both age and PAI estimates. Although this was just a convention, we feel that this was the best what could be done, as there was very little data on error rates of the techniques used.

We used empty puparia of *Protophormia terraenovae*, empty puparia

of *Sarcophaga argyrostoma* (with and without traces of *Nasonia* eclosion), live puparium of *Stearibia nigriceps* and exuvia of *N. littoralis* (Table 3, Fig. 2).

While estimating age at eclosion of *P. terraenovae*, we used developmental data from Grassberger and Reiter [25]. Because puparia were empty at the moment of sampling and there was no indication when adult flies had emerged, age at eclosion was estimated using average daily temperatures prevailing in the room in July (19,65 °C) and June (19,37 °C). The total immature development of *P. terraenovae* in such temperatures would be 22.6 (± 4.5) days in July and 23.2 (± 4.6) days in June. These times probably largely underestimate the true PMI due to the unknown post-eclosion interval and PAI. The same technique was applied for empty but intact puparia of *S. argyrostoma*. Using developmental data of Grassberger and Reiter [26], we estimated that in July its age at eclosion would be 32.2 (± 6.4) days and in June 32.9 (± 6.6) days.

For puparia of *S. argyrostoma* with traces of *Nasonia* eclosion, we used data from Grassberger and Reiter [26] and Grassberger and Frank [27]. First, we estimated the time needed by *S. argyrostoma* to pupariate i.e., 12.6 (± 2.5) days in July and 12.9 (± 2.6) days in June. We assumed that *N. vitripennis* oviposits within 24–30 h after the pupariation of the fly [27], hence one day was added to the estimate. Then, we estimated the total immature development of *N. vitripennis* within the puparium of the fly and got 23.3 (± 4.7) days for July and 24 (± 4.8) days for June. The total age estimates were 36.9 (± 7.4) days for July and 37.9 (± 7.6) days for June. Due to the unknown post-eclosion interval and PAI, the true PMI was larger.

Next, we focused on puparium of *S. nigriceps* (sampled alive at the morgue on August 8th). After 4 days and 16 h of laboratory rearing at 20 °C, the adult emerged. Using data from Marchenko [20], we calculated that age of puparium at the sampling was 370.5 degree-days (DD), (434 DD after Marchenko [20] minus 63.5 DD accumulated in the laboratory). Calculating backwards from August 6th (during August 7th and 8th the body has been stored at the morgue in low temperatures

Table 3
PMI estimation based on insect evidence.

Evidence	PMI estimate [days]	Major sources of error
Empty puparia of <i>Protophormia terraenovae</i>	22.6 ^a 23.2 ^b	Age estimation error (days: $\pm 4.5^a$ or 4.6^b); unknown post-eclosion interval and PAI
Empty puparia of <i>Sarcophaga argyrostoma</i>	32.2 ^a 32.9 ^b	Age estimation error (days: $\pm 6.4^a$ or 6.6^b); unknown post-eclosion interval and PAI
Empty puparia of <i>Sarcophaga argyrostoma</i> with traces of <i>Nasonia</i> eclosion	36.9 ^a 37.9 ^b	Age estimation error (days: $\pm 7.4^a$ or 7.6^b); unknown post-eclosion interval and PAI
Puparium of <i>Stearibia nigriceps</i>	37	Age and PAI estimation errors (± 7.4 days); absence of first colonizers (extra 4–20 days for PMI)
Exuvia of second instar larvae of <i>Necrodes littoralis</i>	20.2 ^a 20.6 ^b	Age and PAI estimation errors (days: $\pm 4^a$ or 4.1^b), unknown post-ecdysis interval

^a Estimations using July temperatures.

^b Estimations using June temperatures.

preventing insect development), the oviposition occurred most likely on July 11th (age of the puparium at sampling was 27 ± 5.4 days). Pre-oviposition interval was estimated using PAI models from Matuszewski et al. [17]. The predictor temperature was assumed to be 19 °C (the average temperature in the building across the mean seasonal pre-oviposition interval of 9 days starting from July 11th), the estimated PAI was 10 ± 2 days. Iterations did not improve the estimate. Total PMI estimate was 37 ± 7.4 days. It underestimated the true PMI, as there were also empty puparia of Piophilidae sampled from the body. They were however very few, a single puparium was sampled on August 8th and three additional empty puparia were sampled on August 11th. Because there were many third instar larval Piophilidae on the cadaver, we assumed that eclosion of Piophilidae had just started. The error resulting from the use of subsequent puparia of Piophilidae (not the first present on the body) was not large, probably no more than 4–5 days. *Stearibia nigriceps* may, however, oviposit for a long time (4 up to 20 days in natural outdoor habitats, unpublished data), so this error might be larger.

The last pieces of insect evidence were second instar exuvia of *N. littoralis*. Using data from Dekeirsschieter [28], age at the second ecdysis of *N. littoralis* was estimated for July at 8 ± 1.6 days and for June at 8.2 ± 1.6 days. Using PAI models from Matuszewski and Szafabowicz [29], the larval PAI of *N. littoralis* was estimated for July at 12.2 ± 2.4 days and for June at 12.4 ± 2.5 days. The total PMI would therefore be 20.2 ± 4 days for July and 20.6 ± 4.1 days for June. The true PMI was longer due to the unknown post-ecdysis interval. Because there were no third instar larvae of *N. littoralis* on the cadaver, we knew that the post-ecdysis interval was very long.

Concluding, puparia of *S. nigriceps* indicated the largest PMI (Table 3), hence this evidence was the most informative, resulting in the final PMI estimate (Fig. 4). Empty puparia of *S. argyrostoma* with traces of *Nasonia* eclosion corroborated the PMI estimate (Fig. 4). The other insects indicated shorter but consistent PMI (Table 3, Fig. 4).

3. Discussion

In general, the habitat encountered in this study posed more difficulties than a typical indoor habitat.

Thermal conditions were more variable; accordingly, we had to use weather station temperature records and the records had to be corrected backwards. Although there are controversies over the use of retrospectively corrected weather station temperatures in PMI estimation [30–33], here this protocol was advantageous. Due to the small daily temperature variation in a typical indoor habitat [12], the use of weather station temperature records is usually pointless there. The quasi-indoor habitats are different, as the thermal conditions may be quite variable in such habitats. Accordingly, the use of corrected weather station temperatures should be the standard protocol for entomologists in such cases.

The cadaver entomofauna was more abundant and diverse as

compared to the typical indoor habitat, and therefore gave prospects for more entomological analyses. Experimental studies usually revealed very few beetles on indoor cadavers [6, 8, 9, 11], similarly previous case reports indicated very infrequent presence of beetles on bodies decomposing indoor [1–4, 7, 10]. Here, the beetle fauna was diverse and this may be frequent in the quasi-indoor habitats. However, the colonization phases of insect taxa were prolonged due to the slower decomposition, which is a rule in indoor habitats [8]. The case analysis was therefore more complex. Insects used for PMI estimation were difficult to be qualified as to the timing of their colonization. Moreover, longer persistence of particular species on the cadaver resulted in a more aggregated assemblage of insects. We sampled several insects which usually do not co-occur on carrion, e.g. larval blow flies and puparia of *Stearibia* or larvae of *Necrobia* [34]. The aggregated colonization increases the risk that entomologist may base the PMI on less informative species, i.e. early colonizer with extraordinarily prolonged colonization phase, e.g. larval blow flies in this case (Table 1). Accordingly, a caution is required, especially when insect samples were taken by entomologically unskilled law enforcement officers.

Another difficulty encountered here was the possibility that some insects sampled in the building were not associated with the body, but came from the rubbish present inside. For this reason we decided not to use Muscidae or Dermestidae in PMI estimation, as they are regular colonizers of organic rubbish.

Most shortcomings of the long PMI estimation occurred in this case. Although the cadaver entomofauna was abundant and diverse, and so could stimulate the conventional succession-based estimation of PMI [35–38], lack of reference data for indoor cadavers excluded such estimation. Moreover, shortages of reference successional data has hindered the choice for the best insects to be used in development-based estimations. The best species is the one that colonizes cadavers later than all the other insects sampled. We have assumed that this would be *S. nigriceps* or species of *Necrobia*, however no experimental data supported this assumption. Another limitation was the absence of robust developmental data for most late colonizing species, e.g. species of *Necrobia*. Finally, although some techniques are being developed [39, 40], at present there is no method useful to estimate the post-eclosion or post-ecdysis interval. Such method could, however, largely expand the timespan for which PMI could be accurately estimated based on insect evidence.

Lastly, the true PMI has not been determined in this case. Therefore, we cannot judge the accuracy of our estimates. Although, there are some data on the accuracy of particular entomological techniques, we are not aware of any data on performance of entomological techniques or methods for the estimation of PMI and this shortage is one of the most important challenges for forensic entomology.

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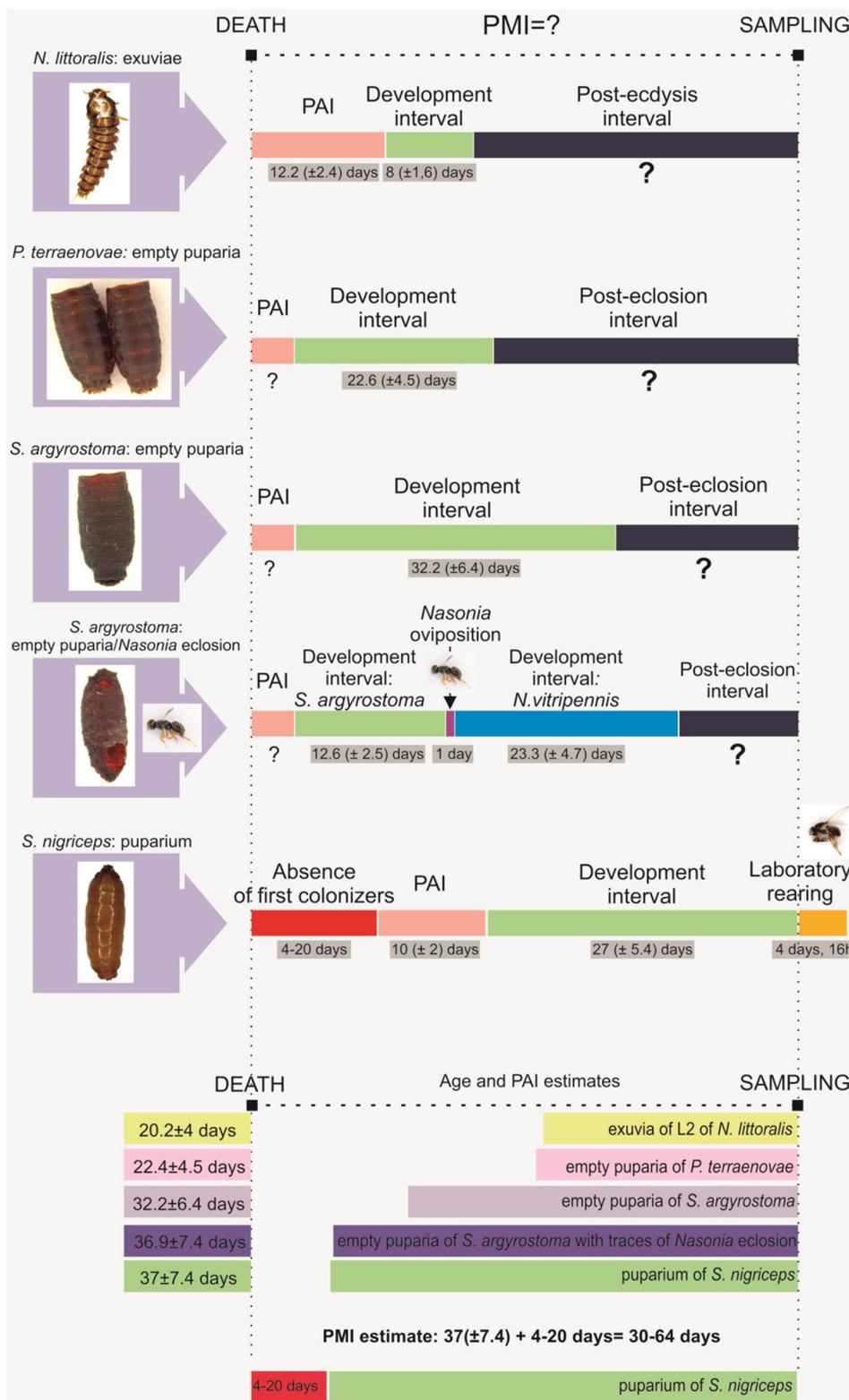


Fig. 4. The graphical summary of the PMI estimation procedure used in this case and associated uncertainty.

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