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PROTOPHORMIA TERRAENOVAE (ROBINEAU-DESVOIDY, 1830) (DIPTERA, CALLIPHORIDAE) A NEW FORENSIC INDICATOR TO SOUTH-WESTERN EUROPE

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Abstract: *Protophormia terraenovae* larvae are found frequently on corpses in central and northern Europe but are scarce in the Mediterranean area. We present the first case in the Iberian Peninsula where *P. terraenovae* was captured during autopsies in Madrid (Spain). In the corpse other necrophagous flies were found, *Lucilia sericata, Chrysomya albiceps* and *Sarcophaga argyrostoma.* To calculate the *posmortem* interval, the life cycle of P. terraenovae was studied at constant temperature, room laboratory and natural fluctuating conditions. The total developmental time was 16.61±0.09 days, 16.75±4.99 days in the two first cases. In natural conditions, developmental time varied between 31.22±0.07 days (average temperature: 15.6ºC), 15.58±0.08 days (average temperature: 21.5ºC) and 14.9±0.10 days (average temperature: 23.5ºC). Forensic importance and the implications of other necrophagous Diptera presence is also discussed.

***Key words*:** Calliphoridae, forensic entomology, accumulated degrees days, fluctuating temperatures, competition, *postmortem* interval, Spain.

Resumen: Las larvas de *Protophormia terraenovae* se encuentran con frecuencia asociadas a cadáveres en el centro y norte de Europa pero son raras en el área Mediterránea. Presentamos el primer caso en la Península Ibérica don-

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de se han recolectado larvas de *P. terraenovae* en autopsias efectuadas en Madrid (Spain). Otras especies necrófagas fueron recolectadas del cadáver, *Lucilia sericata, Chrysomya albiceps* y *Sarcophaga argyrostoma*. Para estimar el intervalo *postmortem*, se estudió el ciclo biológico de *P. terraenovae* a temperatura constante, en condiciones de laboratorio y bajo condiciones naturales variables. El tiempo total de desarrollo fue 16.61±0.09 días, 16.75±4.99 días para los dos primeros casos. En condiciones naturales, el tiempo total de desarrollo varió entre 31.22±0.07 días (temperatura media: 15.6ºC), 15.58±0.08 días (temperatura media: 21.5ºC) y 14.9±0.10 días (temperatura media: 23.5ºC). Se discuten tanto la importancia forense como las implicaciones de otros dípteros necrófagos presentes en el estudio.

Palabras clave: Calliphoridae, entomología forense, grados día acumulados, temperaturas variables, competición, intervalo *postmortem*, España.

1. INTRODUCTION

Two species of the genus *Protophormia* are present in Europe but only *Protophormia terraenovae* (Robineau-Desvoidy, 1830) is found in the Iberian Peninsula (Rognes, 1991). *Protophormia terraenovae* is generally described as a cold climate blowfly, which can be found from boreal to subtropical habitats in all Holartic regions, but it has become established in other continents. Indeed, in 1999 it was reported for the first time in South America (Mariluis, 1999). In Europe, it is common in northern and central countries (Benecke, 1998, Grassberger & Frank, 2004). In the Mediterranean countries, records of this species are very scarce. In Italy, it has been reported from human corpses (Introna *et al.,* 1998). In Portugal, it has been captured on pig carrion (Arnaldos *et al.,* 2006). In Spain, *P. terraenovae* was last recorded in 1958 from the Pyrenees mountains (Viella, Spain) (González-Mora & Peris, 1988) and has been absent in recent studies on sarcosaprophagous communities (Castillo, 2002, Martínez-Sánchez, 2003, Arnaldos *et al.,* 2006) and forensic reports from the Iberian Peninsula.

This species has been used as entomological evidence in criminal investigations to determine the postmortem interval and it is also a serious parasite of cattle, sheep and some wild mammals (James, 1947, Introna *et al.,* 1998, Marchenko, 2001). Despite many aspects of its development time having been studied in Europe (Grassberger & Reiter, 2002) some parameters could vary considering different geographical areas (Martínez-Sánchez *et al.,* 2007). Blowfly species do not display the same growth rates as seen in other geographic zones, and therefore, research should be undertaken on the same species in different regions.

An option to utilize *P. terraenovae* as forensic indicator may be considered the use of isomorphen and isomegalen diagrams to determine minimum development time (Grassberger & Reiter, 2001, 2002) or to calcu-

late the accumulated degrees days (ADD). These methods imply that development under fluctuating temperatures corresponds to the development under the resulting mean daily temperature (Higley & Haskell, 2001), but they can be used readily to determine the development stage of any located species when development occurs at constant temperatures. Clarkson and collaborators (Clarkson *et al.,* 2004) raised *P. terraenovae* under outdoor fluctuating temperatures, and compared the development at a mean constant temperature of 20°C. Recently, development of larvae of different forensically important flies under constant temperatures and under daily fluctuating temperatures in climatic chamber has been investigated and compared (Niederegger *et al.,* 2010). Nonetheless, studies on which development under both constant and fluctuating temperatures are not frequent.

In this study, the first case of *P. terraenovae* from a human corpse in Spain is reported. In addition, the survival of this species was studied, analysing different ambient conditions and competition with other necrophagous species. Firstly, experiments were carried out to obtain biological data about the rate of development of *P. terraenovae* under controlled and outdoor conditions and considerations about their influence on postmortem interval (PMI) estimation. Secondly, data about interaction with necrophagous species is analysed. So, *Calliphora vicina* Robineau-Desvoidy, 1830 shows biological similarities with *P. terranovae*; both species are original from Holartic region but have widespread to other regions during the last years, both are abundant in the diptera sarcosaprofagous community, and both are forensic indicators in corpses; moreover both show annual activity peaks in cold months, so they could be found at corpses at the same time.

2. MATERIALS AND METHODS

Case report

On 12th June 2005, a man and a woman (70 and 75 years old, respectively) were found dead at home, in a central flat of Madrid city, Spain (40.24º N, 3.41º W). The two corpses were found in a room, apparently in a state of natural death. The man was lying on the bed and the woman was sitting in a chair in front of the bed. The authorities were advised and the corpses were taken to the Forensic Anatomy Institute of Madrid where autopsies were carried out on 13th June. The man corpse exhibited colliquative necrosis or black putrefaction, while the woman corpse presented the emphysematous or putrefaction stage. Forensic entomology was not routinely used on scene at that time; therefore no entomological evidence was collected there. Outdoor temperatures were registered from 12 June until the death date estimated by the pathologist (30 May), the maximum being 35.5ºC and the minimum 15ºC; average indoor temperatures were estimated at 24-26ºC. Entomological evidence was collected during autopsies and

specimens preserved by immersing in hot water and storage in 70% ethanol. The specimens were identified (Velásquez *et al.*, 2010) and compared with the immature collection of the University of Alicante (CEUA-Entomological Collection of University of Alicante). Isomorphen diagrams and growth curves were used in order to estimate PMI (Grassberger & Reiter, 2001, 2002, Grassberger *et al.*, 2003, Donovan *et al.*, 2006).

Development at constant and fluctuating temperatures

To study the life cycle of P. terraenovae, three rearing cages (40 x 40 x 40 cm) with about fifty specimens each, 25 males and 25 females, were placed under different temperature situations: at constant temperature i.e.: climatic chamber (Tª 25±0.5°C, RH 50±10%), at room temperature i.e.: lab-conditions (Tªmax: 26.34°C, Tªmin: 23.24°C, Tª average: 24.94°C; RH max-min: 63%-19.6%, RH average: 44.17%) and at fluctuating conditions i.e.: outdoor conditions (see Table 1). All temperatures were recorded every hour by datalogger (HOBO). When adults emerged, fresh liver was introduced into the cages to enable them to mature and to start laying eggs during their first 96 hours. Minimal developmental times for eggs and larva stages during at least three generations were recorded from the first specimen found. To obtain the minimal period in pupation, the 50 first prepupae were individualised and recorded as average and standard errors. Pupal survival rates in three generations in climatic chamber and outdoor conditions were calculated from the first 50 prepupae collected. From these experiments, temperature and minimum period to adult was used to determine the degree-days (DD) or thermal constant (K) in constant conditions (Greenberg & Kunich 2002). K was calculated from the equation $K=y(t-t_L)$, where y is the developmental time (days), t is the rearing temperature (ºC) and t_L is the lower threshold temperature (ºC). Calculations with an inappropriate base temperature (t_L) will overestimate the DD, and so the forensic entomologist may give a false postmortem interval. So, base temperatures for common dipteran species may have to be predetermined or based on geographical proximity (Gennard, 2007). Based on the unknown-origin of our colony of *P. terraenovae* (fish bait bought in Spain), we decided to calculate the DD with minimal temperature values reported for this species [t_L=10ºC (Higley & Haskell, 2001, USA), 8.95 (Grassberger & Reiter, 2002, Europe), and 7.8ºC (Marchenko, 2001, Russia)].

Interspecific competition

Newly enclosed first stage larvae (± 2h old) of *P. terraenovae* (fish-bait origin) and *C. vicina* (Alicante origin) were obtained from laboratory cultures maintained at the University of Alicante (Spain). Larvae were maintained in a controlled room with a temperature of 23±5 °C and relative humidity of 60±5%. Single species groups or pure cultures of 50, 100, 200 and 400 newly hatched larvae were placed in 20 ml glass vials containing 15 g of pig liver. In

the case of mixed cultures, initial densities of larvae were 25 larvae *P. terraenovae* plus 25 larvae of *C. vicina*, 50 plus 50, 100 plus 100, and 200 plus 200, with the same methodology as in pure cultures. Every vial was then placed in a plastic pot (568 ml) containing a 1 cm layer of sawdust in which wandering larvae could pupariate. Pots were sealed with a fine mesh cover and maintained in the same conditions as the colonies. All pots were checked every day and each density was replicated three times, except 50 larvae density where there were five replicates. Time to the first pupa and adult was noted; dead pupae and adult flies were counted and sexed. Once all individuals had emerged, an index of the size (measurement of the vein dm-cu right wing using a binocular microscope fitted with an eyepiece reticule) of all individuals from each replicate was recorded as a measure of the effect of larval competition (Smith & Wall, 1997). To determine the possible differences in the size of individuals and in mortality rates, parametric tests were used, ANOVA in the case of multiple samples and t-test for comparison of two samples, because data were normal and homogeneity of variances. In the cases where assumptions of normality did not meet, nonparametric test U Mann-Whitney was used. When P value greater than 0.05 were discriminated. We used the statistical application SPSS.

3. RESULTS

Case report

Larvae and pupae of Calliphoridae and Sarcophagidae (Diptera) and adults of Histeridae (Coleoptera) were recovered from corpses. A single pupa of *P. terraenovae* was identified by its typical anal papillae, strongly developed with a mixture of spines monocuspid, bicuspid and multicuspid (Velásquez *et al.,* 2010). This is the first time that this species is recorded from human corpses in the Iberian Peninsula. In the corpse of the man, close to *P. terraenovae,* pupae of *Lucilia sericata* (Meigen, 1826), mature larvae of *Chrysomya albiceps* (Wiedemann, 1819) and second and third instar larvae of *Sarcophaga argyrostoma* (Robineau-Desvoidy, 1830) were recovered. In the corpse of the woman, mature larvae of *L. sericata, C. vicina* and *S. argyrostoma* were obtained.

To calculate PMI, pupae of *L. sericata* and *P. terraenovae* were used in the case of the man. Time from oviposition to pupation, the isomorphen diagram for *P. terraenovae* and *L. sericata* gave 10 days and 8 days respectively at 24ºC. In the case of the woman, larvae of *L. sericata* and *C. vicina* were measured (both 1.4 mm) as well as larvae of *S. argyrostoma* (1.8 mm), the last one most abundant in the sample. These values were plotted against time for 24-26ºC in growth curves. So, maximum time from hatching to reach this length was 4 days for *L. sericata,* ~3 for *C. vicina* and ~2 for *S. argyrostoma.* These results are compatible to postmortem minimal data calculated by the pathologist, 12 days for the man (30 May) and 5 days (6 June) for the woman.

Influence of temperature on the development

Under constant temperature conditions the complete life cycle of *P. terraenovae* took 16.61±0.09 days, with the period as larvae 8.33±0.04 and pupae 7.27±0.08 (Table 1). Transformation into DD results in 249.15 with a lower threshold temperature of 10ºC and 266.6 degrees-days with a lower threshold temperature of 8.95ºC. In lab conditions the average total immature period was 16.75±4.99 days, but this varied according to the four generations studied (Fig. 1). Larval period was 8.25±3.20 days, more variable than pupal period, 7.50±1.91 days. In external conditions with fluctuating temperature, the results are different for each generation; the first generation (F1) shows a complete development time of 31.22±0.07 days (average temperature 15.6ºC) (Table 1). After that and probably related to an increase in outdoor temperatures (average temperature 21.5ºC), duration decreases considerably down to 15.58±0.08 days for the second generation (F2) and keeps decreasing in the third generation (F3) to 14.9±0.10 days (average temperature 23.5ºC) (Table 1). The larvae stage required a maximum of 20 days and pupa 10.22 days during F1 (end of April to early of May), and a minimum of 8 and 5.91 days in larva and pupa respectively, during F3 (June to early July) (Table 1). The egg-stage duration was one day in all cases, so this value was added to calculate the duration of the complete life cycle.

Comparing survival of *P. terraenovae* in constant conditions versus outdoor conditions, the percentage of emerged adults in the first case was 100%. However, important variations were observed in outdoor conditions, a 100% rate of survival was only obtained during F1, but during the next generations a decrease in the survival rate was noted related to the increase of temperatures (Table 1).

Table 1: Duration (average and error standard in days) and pupal survival rate of *P. terraenovae.*

AMBIENT CONDITIONS	LARVAL PERIOD	PUPAL PERIOD	TOTAL PERIOD	Survival
	X±SD	X±SD	X±SD	
Climatic chamber (F1, F2, F3) (T: 25±0,5°C)	8.33±0.04	7.27±0.08	16.61±0.09	100%
Lab conditions (F1 to F4) (Average T: 24.9°C)	8.25±3.20	7.50±1.91	16.75±4.99	100%
Outdoor conditions (F1) (Average T: 15.6°C)	20	10.22±0.07	31.22±0.07	100%
Outdoor conditions (F2) (Average T: 21.5°C)	8	6.58±0.08	15.58±0.08	72%
Outdoor conditions (F3) (Average T: 23.5°C)	8	5.91±0.10	14.9±0.10	40%

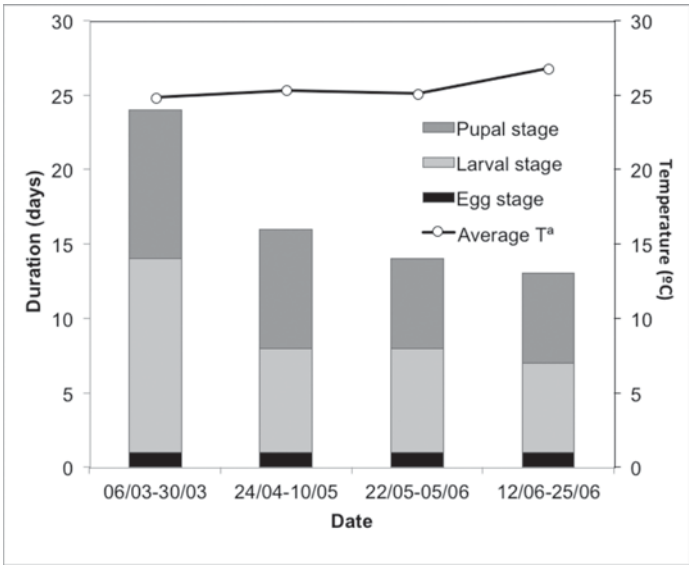

Figure 1: Time of development in egg, larval and pupal stages in several generations of *P. terraenovae* under lab-conditions.

Interspecific competition between *P. terraenovae* and *C. vicina*

Mortality rate increased significantly with increasing initial density in both species and both types of cultures (pure: *P. terraenovae*: F3,24 = 42.99; *C. vicina*: F3,24 = 69.36 and mixed cultures: *P. terraenovae*: F2,9 = 610.34; *C. vicina*: F3,12 = 9.25) (Table 2). Comparing mixed and pure cultures for each species, differences in the mortality rate were observed in lower (50 and 100) and high densities (200 and 400) (Fig. 2). In *P. terraenovae* at low densities, mortality decreased significantly with the presence of *C. vicina* (t=3.35). In *C. vicina* at high densities mortality decreased with the *P. terraenovae* presence (t = 5.46). These results indicate different behaviour in each species: at high density C. vicina is a better competitor and *P. terraenovae* is worse. However at low density, *P. terraenovae* showed an interesting fact, a lower mortality rate in mixed cultures than in pure, contrary to *C. vicina*.

The wing size was analysed by sex separately because females were bigger than males in *C. vicina* (pure: $F_{1,567}$=13.27; mixed: $F_{1,604}$=61.10) and *P. terraenovae* (pure: $F_{1,751}$=40.42; mixed: $F_{1,358}$=11.83). In pure cultures, *P. terraenovae* (males: $F_{3,393}$=257.65; females: $F_{3,358}$ =227) (Fig. 3) and *C. vicina* (males: $F_{3,318}$=352.53; females: $F_{3,249}$=446.83) (Fig. 4) showed significant reductions in the size of wing length with increasing density. In mixed cultures similar results were found. However, these differences were not sig-

Table 2: Mortality rate (%) at initial density of 50, 100, 200 and 400 in pure and mixed cultures of *P. terraenovae* and *C. vicina*.

INITIAL DENSITY	MORTALITY (%)			
	INTRASPECIFIC COMPETITION		INTERSPECIFIC COMPETITION	
	P. TERRAENOVAE	*C. VICINA*	*P. TERRAENOVAE*	*C. VICINA*
50	18.21 ± 10.58	9.33 ± 7.02	4.33 ± 2.31	30.67 ± 2.31
100	29.33 ± 8.08	41.33 ± 5.13	1.33 ± 1.15	34.00 ± 9.16
200	40.33 ± 16.53	76.67 ± 5.51	69.33 ± 5.13	35.67 ± 10.78
400	94.33 ± 0.57	91.33 ± 1.53	100.00 ± 0	57.00 ± 4.58

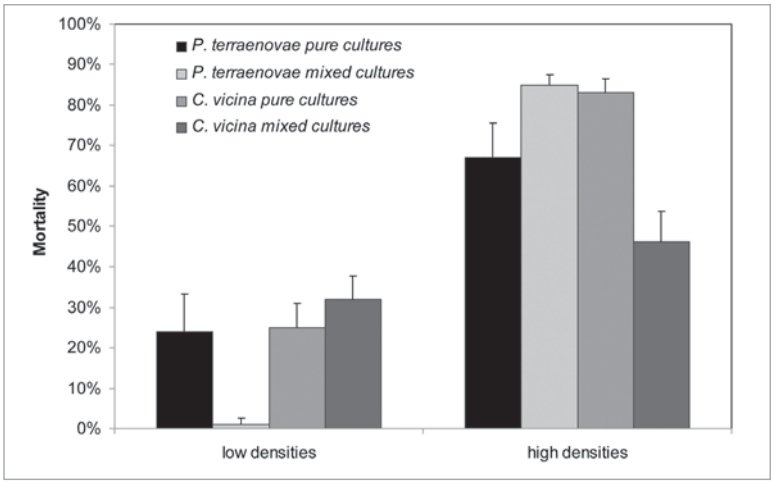

Figure 2: Mortality rate (%) at low densities (50, 100) and high densities (200, 400) in pure and mixed cultures of *P. terraenovae* and *C. vicina*.

nificant between the low density groups (50 and 100) and between the high density groups (200 and 400). In terms of reduced adult size, the data suggest increased levels of competition in mixed rather than in pure cultures of *P. terraenovae* (males U=13603.5; females U=17217.5). The same results were observed in wing sizes of *C. vicina* (males U=4585.5; females U= 8233.5), with larger specimens of pure cultures. In the case of *P. terraenovae,* the effect of the presence of *C. vicina* crops is mainly reflected in densities of 200 (males t=39.27; females t=38,14), and it is obvious in densities of 400 (200 larvae of *C. vicina* and 200 larvae of *P. terraenovae*) where all the larvae died.

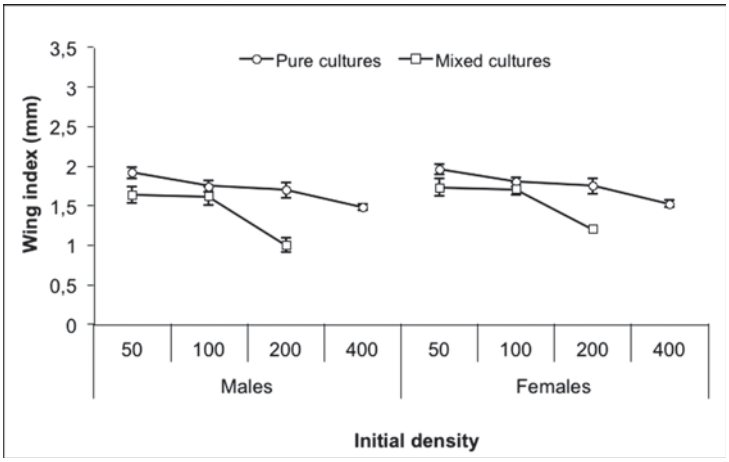

Figure 3: Wing-size index in males and females of *P. terraenovae* in pure and mixed cultures.

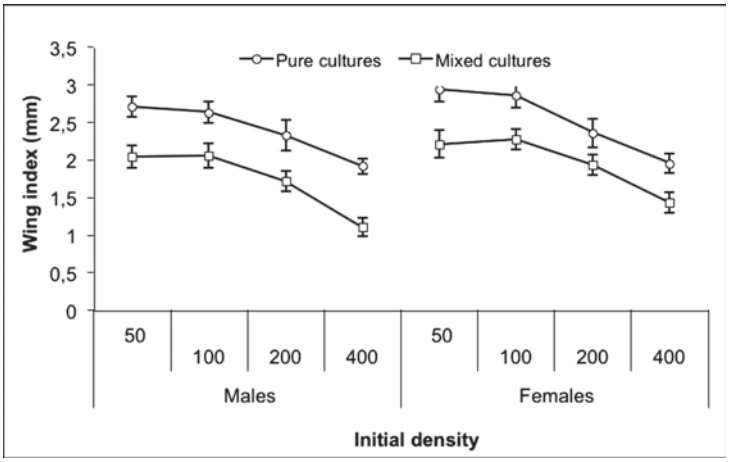

Figure 4: Wing-size index for males and females of *C. vicina* in pure and mixed cultures.

4. DISCUSSION

It is known that *P. terraenovae* is one of the early invaders of carrion and is especially attracted to human cadavers (Nuorteva, 1987) in north and central Holartic regions. However, in South Europe, it was recorded for the first time on a human corpse during August in a rural area of southern Ita-

ly (Introna *et al.,* 1998). In Spain, this species has been previously reported from mountain areas (González-Mora & Peris, 1988). In Alicante (SE, Spain) some specimens have also been captured in mountain areas (unpublished data). In north and central Europe, *P. terranovae* is usually a synanthropic species (Világiová & Peëko, 1994), which agrees with the presence of this species in the case here reported (urban area in Madrid city). This new report confirms its role as entomological evidence and its ecological plasticity. In fact, maximum temperatures for development of *P. terraenovae* are about 35ºC (Greenberg & Tantawi, 1993, Grassberger & Reiter, 2002). However, in latest studies about blowflies in Andorra (Pyrenees mountain) (Martínez-Sánchez *et al.,* 2001) or other Iberian areas (Saloña-Bordas *et al.,* 2009) its presence is at most rare. Carreño *et al.* (2009) propose that *P. terraenovae* is an imported blowflies species commercially available as asticot, so the presence of this species could be related to the use of different species of blowflies and fleshflies as live-bait for fish. This hypothesis might be confirmed by new research and by DNA analysis.

Larvae and adults of *P. terraenovae* are usually present together with other blowflies such as *Calliphora* species and *L. sericata* (Grassberger & Frank, 2004). In carrion, the food in corpses constitutes an important factor that limits the survival of flies, both intra and interspecific survival. Adaptations consist in the rapidity of larval growth and the ability to form viable pupae at a comparatively low final growth weight. But other species such as the 2nd and 3rd instar larvae of *Ch. albiceps* have predatory habits and are therefore able to destroy competitors on the carcass (Ullyett, 1950). The presence of *Ch. albiceps* in the reported case could explain the low number of *P. terraenovae.* Perhaps the absence of samples from the death scene could be the cause of this low number of specimens. However, unlike other blowflies, this species usually puparies on the surface or very close to it, unless the corpse is very wet or exposed to bright light (Nuorteva, 1987, Benecke, 1998).

Entomological evidence was different within the two corpses, and probably indicated different PMI in both cases. While *P. terraenovae, L. sericata, Ch. albiceps* and *S. argyrostoma* were observed on the male corpse, only *L. sericata* and *S. argyrostoma* were collected from the female corpse. Usually, the presence of *Ch. albiceps* is posterior to primary species such as *L. sericata* (Tantawi *et al.,* 1996, Castillo, 2002).

In the place were the corpses were found, temperatures were around 24-26ºC. The minimal time for *P. terraenovae* to reach pupa is 10 days at 25ºC, but for *L. sericata* pupae it is lower, 8 days (Grassberger & Reiter 2001). For this reason, the PMI calculated by the entomologist and pathologist was 12-10 days for the male corpse. For the female corpse, where the biggest larvae were *L. sericata*, the approximation was 4 days using the length of larvae and the development in days of the third stage (oviposition to peak feeding at 24ºC) (Grassberger & Reiter, 2001), compatible with the estimation of the pathologist, 5 days, PMI 5-4 days. *Ch. albiceps* absence

in the woman's corpse and the more advanced development in specimens from the man, confirmed that the man died before the woman.

Grassberger & Reiter (2002) indicate that when temperature is variable, an age range can be estimated in an isomorphen diagram with the maximum and minimum temperature recorded in the rearing place. In *P. terraenovae*, development rates differ between authors and latitudes, but it is generally recognised that larval development starts at 10ºC, increases linearly between 15-30ºC and then decreases (Smith, 1986, Byrd & Castner, 2001, Grassberger & Reiter, 2002). However, holarctic blowfly species could not necessarily exhibit the same growth pattern in different zoogeographic areas. In indoor conditions the use of an isomorphen diagram could provide a quick and precise minimal estimate for the PMI. In our experiments, with development of *P. terraenovae* at constant 25±0,5ºC, the life cycle was 16.61±0.09 days, similar to 15.8 days of Grassberger & Reiter (2002) and 14.6 days of Marchenko (2001) at the same temperatures. When our DD (249.15) are analysed using 10ºC as a lower temperature limit, it is within the range (240.2±9.3DD and 251±0.3DD) given by Grassberger & Reiter (2002). At mild fluctuating temperature indoors, the duration of the life cycle was between 13 and 24 days, in a small range of temperatures. Finally, in outdoor conditions, the rate of total immature development increased with temperature; time from egg to adult varied from a minimum of 14.9 days at average 23.5ºC (in F3), to 31.2 days at average 15.6ºC (in F1). These values are compatible with the range of development time (12 to 38 days for F3; more than 16 days for F1) obtained if maximum and minimum external temperatures are introduced in Grassberger & Reiter's diagram (Grassberger & Reiter, 2002), but it will be too variable to use as legal proof. However, if average temperature is applied to this diagram, results are better (16.5 and 36.5 days respectively).

Rates of development in fluctuating temperatures increase, producing a shorter period of development when compared to constant temperature in *P. terraenovae* (Davies & Ratcliffe, 1994). However, the effect could be opposite at different stages and, while development rates in larval instars increased at constant temperatures, in pupae they decreased (Clarkson *et al.,* 2004). The increase in larval development of *P. terraenovae* under fluctuating temperatures versus constant temperatures may be due to compensation between rapid growth at high temperature and slow growth at low temperature (Niederegger *et al.,* 2010). The duration of the pupal stage is relatively long, representing 43% of the total developmental time (Greenberg & Tantawi, 1993). But larvae duration was longer than pupae, which show a percentage similar to those indicated by Greenberg & Tantawi (1993). At constant temperature larval period was 8.33 days but in the laboratory this was 8.25±3.20 days and outdoors was the most variable, from 8 days to 20 days. However, the pupa period was 7.27 at 25º, in the laboratory it was 7.50±1.91 and in external 10.22 to 5.91 at high temperature. As is frequently indicated, there is a continuing need to refine and improve developmental data of forensically important blowflies. Precise values for min-

imal developmental estimated by stage are important areas of improvement. It is important to notice that when calculating the PMI not only development times at different temperatures are needed but also temperature of the larval mass as well as the photoperiod, as they may influence the development (Niederegger *et al.,* 2010).

The main direct effects of larval competition are, among others, increasing mortality and decreasing size, which in the case of females adversely affect their fertility (Smith & Wall, 1997). Comparing the mortality rate of *P. terraenovae* in pure and mixed cultures, differences were observed, where mortality was higher in pure than in mixed cultures in low densities; however, in high densities the mortality was similar in both cultures. In the case of *C. vicina,* the mortality was higher in mixed than in pure cultures at low densities (≤ 100) and in higher densities mortality was lower in mixed than in pure cultures, this may be because the coexistence with *P. terraenovae* decreases intraspecific competition, increasing interspecific competition. As for size, in all experiments the size of males and females increased in mixed cultures contrary to pure cultures where size decreased with an increase of density.

As it is know, the PMI calculation is of great importance in forensic entomology. For this reason, for their estimation should be taken into account: a) species present in the bodies, since the presence of some of them may influence the development of another. In the case report, the presence of *Ch. albiceps* could have a negative effect on *P. terraenovae* therefore explaining the low number found on the bodies, and in the experiment of competition the presence of *C. vicina* increases mortality in *P. terraenovae,* b) the different temperatures either fluctuating or constant, because they influence positively or negatively the life cycle of the species. Throughout this work, it has been shown that fluctuating temperatures accelerate larval development of *P. terraenovae.*

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