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Discovery of *Graellsia isabelae* (Graells, 1849) in a high-mountain biotope of the Catalan Pyrenees (Spain) (Lepidoptera: Saturniidae)

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Abstract

This work was conducted in two stages separated by approximately 33 years, which is why it is presented in two parts. The first stage took place between 1993 and 1995, within the framework of the author's Doctoral Thesis research (Ylla, 1997). The author transferred 28 cocoons of *Graellsia isabelae* (Graells, 1849) to a forest of *Pinus uncinata* Miller located above the village of Queralbs, at 1600 m a.s.l., (El Ripollès, Gerona, Catalonia, Spain). The objective was to test the hypothesis that "the pupa of *G. isabelae* cannot withstand the cold winter temperatures typical of this ecosystem". If verified, this would explain why the species has failed to establish stable populations in high-altitude *P. uncinata* forests. However, the results did not support the hypothesis. Thirty years later, the situation remains unchanged: *G. isabelae* has still not been detected in *P. uncinata* forests. It will likely be necessary to repeat the study over several additional seasons in order to obtain a more robust dataset. This new research has been carried out in 2026, and the last results are presented in the second part of this paper.
K**eywords**: Lepidoptera, Saturniidae**,** *Graellsia isabelae, Pinus uncinata*, supercooling point, larval development, altitudinal distribution, host plant suitability, pheromone, Spain.

Descubrimiento de *Graellsia isabelae* (Graells, 1849) en un biotopo de alta montaña en el Pirineo catalán (España) (Lepidoptera: Saturniidae)

Este trabajo se ha realizado en dos etapas separadas entre si unos 33 años, siendo este el motivo de que se presente dividido en dos partes. La primera parte tuvo lugar entre 1993 y 1995, en el marco de las investigaciones de su Tesis Doctoral (Ylla, 1997). El autor transportó 28 capullos de *Graellsia isabelae* (Graells, 1849) a un bosque de *Pinus uncinata* Miller situado en las cercanías del pueblo de Queralbs, a 1.600 m de altitud, (El Ripollès, Gerona, Cataluña, España). El objetivo era contrastar la hipótesis siguiente: "las crisálidas de *G. isabelae* no pueden soportar las frías temperaturas hibernales propias de este ecosistema". En caso de confirmarse, ello hubiera explicado porqué dicha especie no ha conseguido establecer poblaciones en estos bosques de *P. uncinata* propios de altitudes elevadas. Los resultados no confirmaron la hipótesis, por lo que 30 años después, la situación sigue sin cambios: *G. isabelae* no ha sido detectada aún en los pinares de *P. uncinata*, llegándose a la conclusión de que probablemente será necesario repetir el trabajo de campo unas cuantas temporadas más, llevando a cabo más y mejores muestreos. Esta nueva investigación ha sido llevada a cabo en 2026, presentando los últimos resultados en la segunda parte del presente artículo.
Palabras clave: Lepidoptera, Saturniidae, *Graellsia isabelae*; *Pinus uncinata*; "supercooling point"; desarrollo larval; distribución altitudinal, plantas huésped propicias, feromona, España.

Introduction

It is well established among entomologists that *Pinus sylvestris* L. and *Pinus nigra salzmannii* Dunal, are the only known host plants utilized by *Graellsia isabelae* (Graells, 1849) under natural conditions. Aside from these two conifer species, no larvae have ever been observed feeding in the wild on any of the other conifer species that occur throughout the Iberian Peninsula.

Pinus uncinata Miller*,* or black pine, is a species of conifer in the Pinaceae family native to the high European mountains, where it lives in the subalpine stage. It is found mainly in the Pyrenees, Alps, Carpathians and Balkans. It forms extensive forest stands within the subalpine belt of the Pyrenees, typically between 1,700 and 2,400 metres a.s.l., where it defines the characteristic plant community *Rhododendron uncinatae typicum* (Folch, 1981).

Besides the Pyrenees, *Pinus uncinata* is restricted to a few isolated populations within the Iberian System, notably in Sierra Cebollera (Soria and La Rioja) and Sierra de Gúdar (Teruel), the latter representing the southernmost limit of its distribution. Notably, *G. isabelae* has not been recorded in any of these locations.

A similar situation is those which occur in the extensive forests of *P. sylvestris* located in the Northern Iberian System (Soria, Segovia, Burgos, La Rioja) in which there is also no evidence of its presence. Chefaoui & Lobo (2007) also indicate other areas, potentially suitable for *G. isabelae*, but where it has not yet been found (mountains of Cantabria, Zamora and Galicia).

It is worth highlighting that, in the Pyrenees, *P. uncinata* extends well into the alpine zone and, conversely, also penetrates the montane belt, where it coexists with *P. sylvestris*, occasionally descending in isolated individuals into shaded ravines as low as 1,000 metres or even lower (Vigo, 1976).

So, ¿why has this species not colonized habitats where this pine is the characteristic arboreal species? The altitudinal and climatic range in which this hardy pine grows may lie beyond the cold tolerance of the moth's immature stages, which could explain why there is no recent evidence of naturally occurring colonies feeding on that pine species (Monasterio et al. 2017; González-Castellano et al. 2023).

Many other researchers have reached the conclusion that, under natural conditions, only two trophic substrates are currently documented for *Graellsia isabelae*: *Pinus sylvestris* and *Pinus laricio salzmannii* (Ylla, 1997), De Arce-Crespo et al. 2010; Baixeras, 2001; Romo et al. 2012; Sánchez-Fernández & De Arce-Crespo, 2017; Marí-Mena et al. 2018; González-Castellano et al. 2023). There is even one study that proves a direct relation among *G. isabelae* and *P. sylvestris* and *P. laricio* (Chefaoui & Lobo, 2007). Nässig, 1991, demonstrated the acceptance of *P. mugo* Turra, but it was a captive breeding experience.

In laboratory rearing experiments where *G. isabelae* larvae were offered different host plants, the tested species could be grouped into the following three categories based on larval development success:

1. Clearly toxic species. Larvae fail to complete the first instar (L1) and die prematurely. Examples: *Pinus halepensis* Mill*., P. pinea* L*., Larix spp., Abies spp.* and *Picea spp.*
2. Partially suitable species. Some larvae successfully pupate, and a few adults may emerge. Examples: *P. pinaster* Ait and *Cedrus spp.*
3. Highly suitable species. These species are well accepted under captive conditions, yielding developmental results comparable to those obtained with *Pinus sylvestris*. Example*: P. uncinata.*

To date, no natural populations of *G. isabelae* have been found feeding on any of the eight former species mentioned in categories 1, 2 and 3.

Regarding the use of *P. uncinata* as a good trophic substrate, Ylla (1997) experiments clearly demonstrated that this pine is a perfectly suitable trophic substrate for the proper development of *Graellsia isabelae*. The results obtained were comparable to, and in some cases exceeded, those recorded for *Pinus sylvestris*, with pupation rates reaching up to 50%.

Several factors may help explain the absence of *Graellsia isabelae* in *Pinus uncinata* forests of the Pyrenees. First, the ecological requirements of the species may not be fully met in these habitats, despite their apparent suitability in terms of altitude and temperature. For example, although *P. uncinata* is a valid trophic substrate, it may not provide the same chemical, nutritional or structural properties as *P. sylvestris*, which is known to be the preferred host plant. In addition, the fragmented distribution and limited dispersal ability of *G. isabelae* could hinder its colonization of these isolated high-altitude forests. Historical biogeographical factors

and postglacial recolonization patterns may also have played a role, preventing the species from reaching certain mountain areas. Lastly, biotic interactions, such as the presence of predators, parasitoids, or the absence of mutualistic relationships, could further contribute to its absence from these ecosystems.

Some more questions to take in consideration are:

1. Insufficient field surveys at the appropriate time of year. This is something that has to be considered. The author has conducted repeated and targeted sampling in subalpine pine forests in the Ripollès region, employing light traps and even virgin females during the species' flight period, but without obtaining any positive detections.
2. Winter temperatures: are low winter temperatures the cause of *Graellsia*'s failure to colonize *Pinus uncinata* forests? The extremely low winter temperature could potentially affect the pupal stage, as this is the overwintering phase during which *G. isabelae* would be exposed to low temperatures possibly below the supercooling point.

In temperate regions, insects survive the winter in a frozen state (freezing-tolerant species) or prevent freezing through the synthesis of antifreeze or cryoprotectant compounds which lower the Supercooling point (freezing-intolerant species). As we will see, *Graellsia* belongs to the second group, with a Supercooling Point in principle sufficient to cope with the expected winter thermal drops at ground level, often covered by a more or less thick layer of snow.

Interestingly, the upper altitudinal limit of *G, isabelae* (approximately 1,700-1,750 m), coincides with the elevation at which *P. uncinata* typically begins to appear. The highest recorded occurrence of the species is at 1,866 m, at the summit of "La Mogorrita" in the Serranía de Cuenca, where the larval host plant is *P. sylvestris* (De Arce-Crespo et al. 2010).

Ibáñez et al. (2008) reported the presence of a population in Sierra María (Almería), where the dominant tree species are *Pinus halepensis* and *Pinus pinaster*. Given that *P. halepensis* is not a viable larval host (larvae die in the first instar), and that captive rearing experiments with *P. pinaster* have shown extremely low success rates (not a single imago was obtained from a total of 79 eggs) (Ylla, 1997), the trophic substrate utilised by the Sierra María population remains uncertain. The possibility of isolated individuals of *P. nigra* in the area cannot be ruled out.

Part One: Considerations on winter temperature as a cause for the absence of *G. isabelae* in the Pyrenees (Spain).

Materials and Methods (first part)

Field data collection took place over two seasons: 1993-1994 and 1994-1995. The same site was chosen for both seasons: a typical *P. uncinata* forest in the "Serra de l'Estremera", Ripollès, near the village of Queralbs (Gerona, Spain). A point near the path to the "Font de l'Home Mort" at an altitude of 1,800 m was selected (Figure 1).

Figure 1. Exact point (see arrow) where the chrysalides spent winter.

The Supercooling Point (SCP) of *G. isabelae* was determine by Ylla (1997) following the method described by Leather et al. (1993). The chilling experiments are described also in Ylla (1997).

The Supercooling Point (SCP) is the lowest temperature an insect can reach before ice inevitably forms. When this threshold is exceeded, freezing occurs suddenly and is almost always lethal if the species is not freezing-tolerant.

Season 1993-1994

On November 23, 1993, a group of 8 chrysalids obtained from captive breeding in the same year were transported to the previously chosen location. After confirming their healthy condition, they were placed inside a sealed plastic container with small holes to allow gas exchange and water drainage. This container was designed to protect the chrysalids from predators such as shrews. All eight chrysalids were male.

The container, filled with pine litter, was closed and camouflaged among the forest litter near the base of a pine tree. A Hamster TWIN temperature logger was placed next to it, programmed to take automatic readings every 30 minutes for 333 days. The entire setup was camouflaged with forest litter and marked with a wooden stake (Figure 2).

Figure 2. Images of the data logger and the plastic box with the chrysalides.

On March 31, 1994, after 128 days, the chrysalids were transferred to the urban area of Serrabonica (Gurb, Barcelona) at 650 m altitude, where they remained under natural conditions until adult emergence.

Control group: Comprised of 8 identical chrysalids that remained in Serrabonica for the same 128-day period.

Season 1994-1995

The same procedure was repeated with a few changes. Three groups of 10 cocoons (male and female) were used: one group was left on the ground, camouflaged with forest litter; another was hung from a branch 1.0 m above ground; and the third was the control group kept in Serrabonica. The mountain site was the same as the previous season. The cocoons were transported on October 27, 1994, and retrieved on April 4, 1995, thus remaining for 159 days.

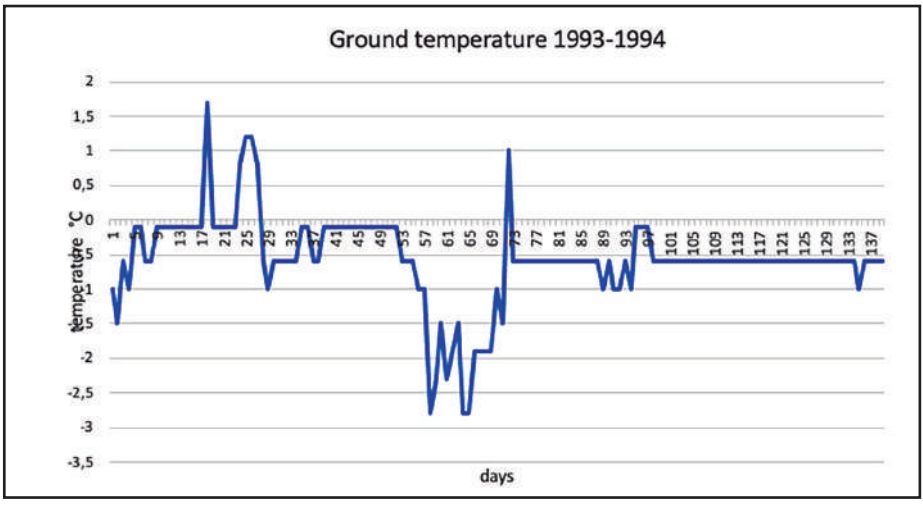
The author wishes to emphasize the antiquity of the dataset which, despite being in many cases more than 30 years old, remains fully valid at the present time. This statement applies unequivocally to all the data and information reported in Ylla (1997). Admittedly, over the past three decades, global knowledge of *G. isabelae* has expanded in several areas. For instance: pheromone identification, improving its geographical distribution, development of predictive distribution models, conservation strategies and population monitoring. Nevertheless, about the specific issue under consideration here, the state of knowledge has essentially remained unchanged.

Results

Figure 3 shows the temperature graph for the 1993-1994 season at the Pyrenean site. Temperatures were

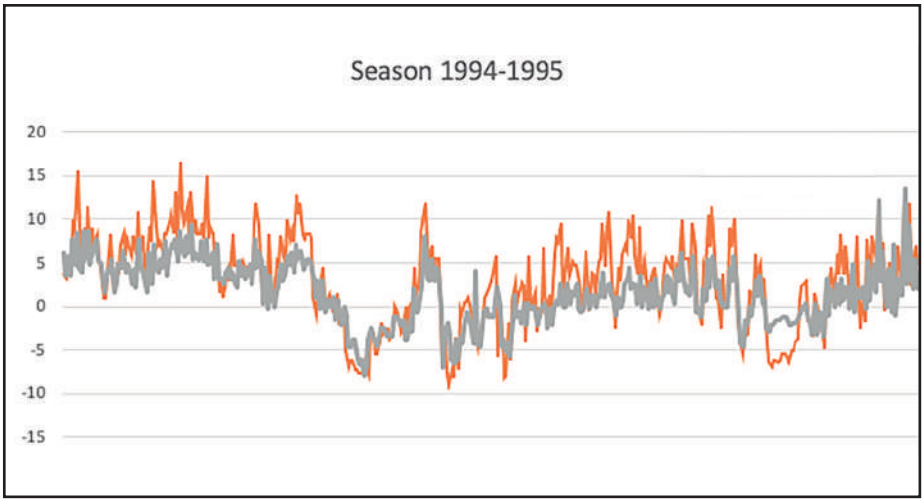
slightly below 0ºC for much of late January and almost all of February. The lowest temperature recorded was -2.8ºC in January 1994.

Figure 3. Temperature graph for the 1993-1994 season.



Thermometric values from the first season were unexpectedly high for an altitude of 1,800 m in the Pyrenees. This becomes clear when compared with the second season's values (Figure 4).

Figure 4. Temperature trend from the 1994–1995 season, with measurements taken at 1.0 meters above ground (in red) and at ground level (in grey).



As expected, air temperatures were more extreme than those recorded at ground level. This implies that the maximum and minimum values correspond to the higher measurement point, whereas the chrysalids, which invariably remain within their cocoons beneath the forest litter, were not directly exposed to such extremes. Table 1 presents the three lowest temperatures recorded at 1.0 m above ground (all from the same season), together with the corresponding ground-level values. These data clearly illustrate the considerable thermal insulation provided by the snow layer.

Table 1. Lowest temperatures recorded at 1.0 m compared to ground temperatures at the same time. These are the three lowest values observed.

Data	Temp. at 1,0 m	Temp. at grown level	Differences
22-XII	-8,3 °C	-5,8 °C	2.5 °C
3-I	-9,0 °C	-3,2 °C	5,8 °C
11-I	-8,3 °C	-3,8 °C	4,5 °C

Table 2. The minimal and maximal monthly two season temperatures and the number of available chrysalids, are registered in Table 3. In the same table are indicated the quantity of pupae available, their location and the quantity of emerged one.

Location of the probes				
Months	**0 m**		**1,0 m**	
	Minimum °C	**Maximum °C**	**Minimum °C**	**Maximum °C**
December 93	- 1,9	+ 5,2	------	------
January 94	- 2,8	+ 1,2	------	------
February 94	- 1,5	- 0,1	------	------
March 94	- 0,6	+ 9,2	------	------
November 94	+ 1,3	+ 9,6	+ 0,3	+ 16,6
December 94	- 7,0	+ 8,3	- 8,3	+ 14,4
January 95	- 7,7	+ 4,2	- 9,0	+ 9,9
February 95	- 5,1	+ 8,0	- 5,8	+ 13,1
March 95	- 5,4	+ 12,2	- 7,0	+ 13,8

Year	Location	Number of chrysalides	Number of chrysalides emerged	%
1994	0 m	8	6	75
1995	0 m	10	9	90
1995	1,0 m	10	7	70
		Total 28	Total 22	78,6

The minimal and maximal monthly two season temperatures and the number of available chrysalids, are registered in Table 2. The same table shows the number of available pupae, their location, and the number of individuals that emerged.

Combining data from different seasons, overwintering in the Pyrenees did not harm the chrysalids. The emergence rate was even higher in the mountain group (78.6%) compared to the control group (66.7%) (Table 3).

Discussion

Supercooling is a physiological and physical phenomenon that many insects use to survive subzero temperatures without freezing. Under normal conditions, water freezes at 0°C, but within insect tissues this only happens if there are ice-nucleating agents (particles or structures that promote the formation of ice

crystals). If insects manage to eliminate or control these nucleators, their body fluids can be cooled below 0ºC without immediately freezing.

Table 3. Comparison, of percentage of emerged pupa between the Pyrenees and Serrabonica.

	Pupae at Pyrenees	**Pupae at Serrabonica**
Total	8 + 10 + 10 = 28	10 + 8 = 18
Emerged	6 + 7 + 9 = 22	9 + 3 =12
% emerged	78,6 %	66,7 %

This means that their body temperature can drop to -20ºC, -30ºC, or even lower, while the fluids remain in a liquid state.

As it has been said before, the lowest temperature an insect can reach before ice inevitably forms are called the supercooling point (SCP). Once this threshold is exceeded, freezing occurs suddenly and is almost always lethal if the species is not freezing-tolerant.

Discussion of insect cold tolerance typically revolves around the paradigm that insects use two main strategies to survive low temperatures: (1) freezing avoidance, in which insects endure sub-zero conditions by preventing the formation of ice crystals within their bodies, and (2) freezing tolerance, in which insects withstand sub-zero conditions by tolerating the presence of ice within their tissues. The degree of freezing tolerance may be classified as partial, moderate, or strong, depending on the amount of ice formed (Sinclair, 1999).

Bale (1996), proposed a new categorisation of the insect cold tolerant group, suggesting five distinct situations, ranging from least to most cold hardy, based upon when mortality occurs.

(1) Opportunistic, species cannot enter a dormant state and die when temperatures are too low to maintain normal metabolism.

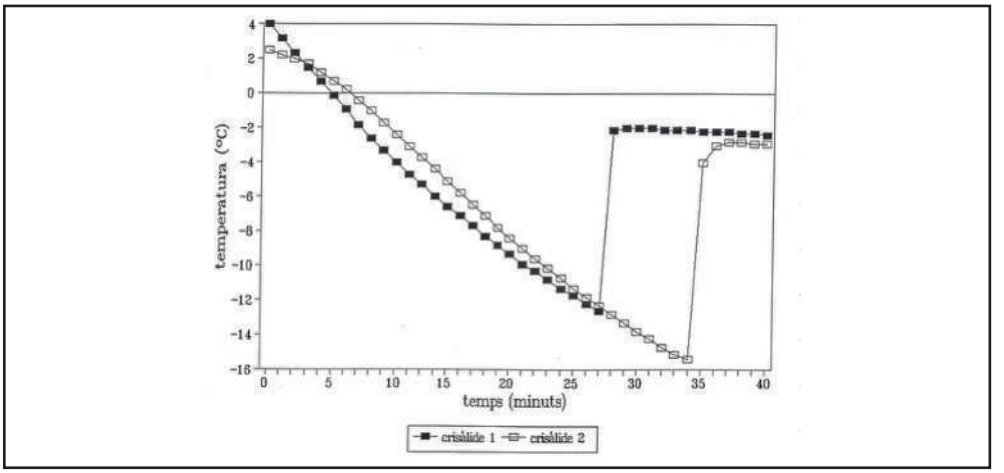
(2) Chill-susceptible, those species that die after brief chilling at moderate to high sub-zero temperatures.

(3) Chill-tolerant, those species that die after prolonged chilling at moderate to low sub-zero temperature above the freezing temperature of SCP.

(4) Freezing avoiding, those that can survive extensive periods in the supercooled state (supercooling), but die when the organism freezes (i.e., at the SCP)

(5) Freezing tolerant, those that can survived the formation of ice in their body tissues.

Figure 5. SCP curve of two *G. isabelae* pupae. Note the sudden temperature rise at the SCP, as a result of the release of the latent heat of crystallization of water. See Ylla (1997) to know how it is performed.



Ylla (1997) showed that 100% of *Graellsia* pupae emerged normally after spending 240 hours (10 days) at -8ºC. However, when the temperature dropped to -10ºC, 80% of the pupae died after 216 hours. Considering these results, *G. isabelae* can be categorized as a chill-tolerant species, corresponding to point 3 of the five previously established physiological categories.

In natural biotope of Pyrenees, at 1,600 m, temperatures of -8ºC to -10ºC are easy to occur, suggesting that the chilling generates by itself a cumulative harmful effect that produced pupal mortality even without freezing.

Supercooling point (SCP), as previously mentioned, represents a thermal threshold which, once reached, in the case of freezing avoiding, results in 100% mortality. Ylla (1997) determined the supercooling point (SCP) using 40 pupae, obtaining an average value of -15.46ºC ± 1,83 (Table 4) with minimum and maximum values of -18.3ºC and -8.2ºC, respectively. Figure 5 presents the supercooling curves of two *G. isabelae* pupae, highlighting the considerable inter-individual variation (approximately 4ºC between the two specimens).

Table 4. Supercooling point determined with 40 pupae.

Average of the Supercooling Point	-15,46°C
Standard deviation	-1,83°C
Minimum value	-18,3°C
Maximum value	-8,2°C

This study confirms that it is possible in the ground litter in the Pyrenees to reach and sustain temperatures below -10ºC, as nearly occurred in the second studied season. In any case, neither of the two possibilities (dying because of SCP or dying because of the chilling) took placed in winters of 1993-1994 and 1994-1995, as was demonstrated next Spring by the emergence of normal imago's.

In the Pyrenees, in neither of the two trials did the temperature fall low enough to reach the supercooling point (SCP). During the first season, it remained close to -2/-3ºC, while in the second season it decreased considerably further. Therefore, the six death chrysalids were not due to freezing, but most likely to chilling injury.

Table 5 presents the SCP values of different moth species for comparison.

Table 5. Examples of SCP of some European and American species.

Species	Supercooling point (SCP)	Observations
Graellsia isabelae (Graells, 1849) (Saturniidae)	from -8ºC to -18ºC; $\bar{x}$ =-15ºC	Ylla (1997)
Mythimna unipuncta (Haworth, 1809) (Noctuidae)	-24,29ºC	Roberts et al. (1972)
Agrotis ypsilon (Hufnagel, 1766) (Noctuidae)	-24,42ºC2	Roberts et al. (1972)
Plodia interpunctella Hübner, [1813] (Pyralidae)	-14 / -16ºC	Carrillo et al. (2005)
Plodia interpunctella Hübner, [1813] (Pyralidae)	-21ºC	
Antheraea pernyi (Guérin-Méneville, 1855) (Saturniidae)	-15,6ºC prediapause -20,1ºC diapause -17,5ºC postdiapause	Liu et al (2016)
Malacosoma disstria Hübner, [1820]) (Lasiocampidae)	From -26,8ºC to -40,3ºC	Uelmen et al. (2016)

Erebia medusa (Fabricius, 1787) (Nymphalidae)	From -10,8ºC to -16,5ºC	Vrba et al. (2022)
Erebia pronoe (Nymphalidae)	From -16,1ºC to -18,7ºC	Vrba et al. (2022)
Gynaephora groenlandica Wocke ex Homeyer, 1874 (Lymantriidae)	-8ºC	Danks et al. (1994)
Chilo supresalis (Walker, 1863) (Pyralidae)	-11ºC	Atapour M (2009)
Pieris brassicae (Linnaeus, 1758) (Pieridae)	-23ºC / -28ºC	Pullin et al. (1991)
Epirrita autumnata (Borkhausen, 1794) (Geometridae)	-35/-36ºC	Tenow & Nilssen (1990); Nilssen & Tenow (1990)

One of the most surprising results is the value of -8ºC recorded for *Gynaeohora. groenlandica* (Wocke ex Homeyer, 1874). This relatively high SCP indicates that it is a freezing tolerant species whose survival depends on its ability to withstand body freezing at comparatively high temperatures, rather than on extreme supercooling capacity. A possible explanation is that achieving and maintaining very low SCP values over long periods may be energetically too costly. Indeed, many Arctic species are freezing-tolerant, surviving despite internal ice formation.

It is therefore clear that having an SCP located at high or very high temperatures is an advantage if you are a freezing-tolerant organism living in cold or very cold environments, since it is energetically less costly to survive frozen than unfrozen.

Among the other species listed in the table, *Graellsia isabelae* shares its habitat with the Noctuidae *Agrotis ypsilon* (Hufnagel, 1766) and *Mythimna unipuncta* (Haworth, 1809), both of which have considerably lower SCPs than *G. isabelae*.

G. *isabelae* seems to be perfectly designed to overcome the low winter temperatures that occur in the high mountains, at ground level, among the fir. For example, the SCP decreases as the seasons progress, and the probability of colder conditions increases. That is, the SCP of just formed pupae, at the end of July, is -12,65ºC, whereas this same parameter at the end of January, when pupae really need to withstand low temperatures, the supercooling point have dropped to -16,43ºC (Ylla, 1997).

Undoubtedly, the behaviour of sheltering from the cold by hiding beneath forest litter and/or under a variable layer of snow also constitutes an advantage for the species.

The frequency with which winter temperatures reach the SCP remains unknown and can only be determined through repeated experimentation. Since winters vary in severity, the two examined here are illustrative only. Long before reaching the SCP, most pupae are likely to die from cumulative cold stress. Should the SCP be reached, all individuals in advanced or developing colonization stages would inevitably perish, forcing the population to restart.

The author emphasizes that this study focuses solely on the potential establishment of new colonies in higher, cooler areas, using *Graellsia* as an example. Nevertheless, the possible effects of summer overheating should not be overlooked, as elevated temperatures may affect the eggs or larvae of *Graellsia* or other species which are generally more sensitive to desiccation. For instance, the eggs of *Graellsia* fail to develop when temperatures reach 30ºC (Ylla, 1997).

Conclusions of the first part

1. Of the 28 pupae of *G. isabelae* that overwintered in the Pyrenees over the two-year period, a 78.6% successfully completed its biological cycle, showing that it is possible for *G. isabelae* pupae to spent winter satisfactorily in the *P. uncinata* forests of the region.
2. During the winters of 1993-94 and 1994-95, neither of the two seasonal minimum temperatures approached the SCP (-15.46ºC), with a minimum of -9.0ºC at 1 m in January 1995 and -7.7ºC at

ground level, also in January 1995.

3. *G. isabelae* is a chill-tolerant species who combines a degree of resistance to freezing (with the SCP as a threshold) with the advantage provided by its behaviour of sheltering from the cold, by hiding beneath the forest litter and/or under a variable layer of snow.
4. It has not been possible to demonstrate mortality of *G. isabelae* due to low winter temperatures, as in neither of the two seasons did temperatures reach the SCP. The 6 chrysalids (27,2%) that died likely did so because of chilling injury.
5. It has been showed that the SCP threshold is not reached every year. Only in exceptionally cold years, which may occur infrequently, the temperature can drop so low as to reach the SCP value.
6. The low SCP value undoubtedly provides strong protection to the pupae of *G. isabelae*, which rarely die from freezing. Moreover, given the standard deviation of the SCP and the distance between the minimum and maximum values, it is likely that some individuals survive.
7. Since winter temperatures have remained well above the SCP threshold (-15.46ºC), any hypothetical populations present would likely have successfully endured the characteristic cold of high-elevation zones.
8. Therefore, it cannot be ruled out that *Graellsia isabelae* could successfully inhabit the same habitat as *P. uncinata* pine forests in the Pyrenees.
9. Considering all the evidence presented (the presence of a suitable host plant, the low SCP of the pupae, winter temperatures remaining above the SCP, and the undeniable effects of climate change) together with intensified surveys, the author thinks that *G. isabelae* could eventually be detected in *Pinus uncinata* forests in the Pyrenees.

Part Two: Confirmation of the presence of *G. isabelae* in the Pyrenees (Spain)

Materials and Methods (part two)

To maximize the likelihood of finding a potential *Graellsia isabelae* population, male-targeted detection methods were essential from the start. Individuals of this sex are much more active than females, especially during the first two hours of darkness. At this time, stimulated by the varying concentration of pheromones released by females, they fly erratically and intensely in search of them.

These flying males can be detected using the following four methods:

1. With a butterfly net and a flashlight. Success is highly unlikely.
2. With synthetic pheromone. Good results.
3. With actinic light traps or other types of light. These usually yield good results.
4. With virgin females in calling position. Good results, but females must be available. Unmated females that are three or four days old have the highest attraction capacity.

In this study, the first three methods were used simultaneously. The light traps were the classic Heath traps, equipped with 8W actinic light and the synthetic pheromone was provided by INRA (Institut National de la Recherche Agronomique). Two vials were borrowed, each containing 4 nanograms of the pheromone synthesized by Jocelyn Millar in 2010 and kept frozen at -20ºC.

The vials containing the pheromone were suspended inside a 15 cm diameter transparent methacrylate cylinder, which was wrapped with a rope whose texture mimicked the female's furry abdomen. Figure 6. The purpose was to confuse the males, which would attach themselves to it, believing they were copulating, as indeed occurred.

In principle, doubts could have arisen regarding the functionality of a pheromone synthesized 16 years ago and custom-made for the French subspecies *G. isabelae galliaegloria* Oberthür, 1922. However, initial trials in *Pinus sylvestris* forests quickly dispelled any uncertainty, hence, it was confirmed that the synthetic French pheromone, after all these years, was still able to attract the Catalan subspecies *G. isabelae paradisea* Marten, 1955.

On May 11, 2026, traps were installed at 1,800 m in a dense *P. uncinata* forest at "Font de l'Home Mort",

in Queralbs (UTM 312TDG28), the same place where, more than 30 years ago, the cocoons were brought to overwinter.

Figure 6. Methacrylate cylinder wrapped with rope, with a pheromone septum suspended inside.

Two pheromone traps and one actinic light trap were placed in a line along approximately 500 m of a forest track that runs parallel to the upper part of the valley carved out by the "Tosa River" Figures 7-8. Sampling lasted for the first two hours of darkness, which is when the males are active.

Figure 7-8. 7. Male "copulating" with the rope. It is one of the three specimens detected at 1800 m. In the background, the needles of *P. uncinata* can be observed. **8**. Site where the three specimens were detected.

In total, three specimens were detected, all males attracted by the pheromone and none by the actinic light.

The fact that three males arrived makes it highly improbable, if not impossible, that they were lost individuals coming from lower down the mountain where *P. sylvestris* is present. Everything indicates that they were individuals belonging to an autochthonous population established in the same habitat as *P. uncinata*.

Conclusions of part two

1. The presence of *G. isabelae* in high-mountain habitats colonized by *P. uncinata* is unequivocally confirmed.
2. As shown in the first part of this study, no factor against the presence of *G. isabelae* at high altitudes was identified, for the simple reason that none exists.
3. *P. uncinata* is definitively confirmed as a new host plant for *Graellsia* under natural conditions.
4. This discovery considerably expands the potential distribution area of *Graellsia*, at least in the Pyrenees.
5. These findings demonstrate that *Graellsia*, as a species, exhibits greater resilience to climate change than previously anticipated. *Pinus uncinata* woodlands occupy an extensive area (110.000 Ha) characterized by a cold climate regime which, functioning as a climate refugium, should enable *G. isabelae* to tolerate the thermal increase that empirical evidence suggests is already underway.

Observation concerning genus

As is evident, the author has consistently included the taxon *isabelae* within the genus *Graellsia* Grote, 1896, a view contrary to that of most current works, in which this taxon is placed within the genus *Actias* Leach, 1815. The use of the binomen *Graellsia isabelae* is advocated based on the validity of the cladistic analysis in which 93 characters (morphological, molecular, and ethological) from 16 species of Saturniidae were compared, leading to the conclusion that the genus *Graellsia* is valid (Ylla et al. 2005).

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Conflict of Interest

The author declares that there are no known financial interest or personal relationships that could have influenced the work presented in this article.

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