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Anatomical study of *Peltigera canina*, *P. membranacea* and *P. praetextata* (lichenized Ascomycotina)

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The anatomical variation of *Peltigera canina* (L.) Willd., *P. membranacea* (Ach.) Nyl. and *P. praetextata* (Sommerf.) Zopf of the *P. canina* group was studied using statistical methods (PCA, DA, ANOVA). Forty four samples were analyzed in order to clarify possible taxonomic implications of spore size, thallus thickness, and of width and height of veins. The thallus thickness between veins and the vein height are the main characters to separate the three species. The relationships in the anatomical characters are closest between *P. canina* and *P. praetextata*.

Key words: Anatomy, Iberian Peninsula, *Peltigera canina*, *P. membranacea*, *P. praetextata*

From the original *Lichen caninus* described by Linnaeus in 1753, several different species have later been separated and included in the *Peltigera canina* group *sensu* Holtan-Hartwig (1993). The species of this group typically lack secondary metabolites, have their veins made up of conglutinated hyphae, and have a smooth and tomentose upper surface (Holtan-Hartwig 1993).

Many species of this group have been studied in different ways in order to elucidate their taxonomical position. Several works clarified the structure and development of the vegetative propagules. They are important taxonomical characters independent from external conditions (Darbishire 1926, Lindahl 1953, 1960), although Thomson (1948) concluded that regeneration lobules could not be used as a taxonomic character to separate *Peltigera praetextata* (Sommerf.) Zopf from related taxa.

In spite of the existence of some "good characters" to separate all species included in the *Peltigera canina* group such as veining pattern and rhizines, some problems remain in the proper identification of *P. canina* (L.) Willd., *P. membranacea* (Ach.) Nyl. and *P. praetextata* because many thalli show intermediate morphological characters. This problem was also underlined recently by several authors (Goffinet & Hasting 1994, Goffinet *et al.* 1994, Vitikainen 1994). As these three species are very common in the Iberian Peninsula (Martínez & Burgaz 1995) the aim of this study is to test if the anatomical characters have a taxonomic value in identifying them. The altitude of collection was used as an independent factor to establish a vertical gradient of the species.

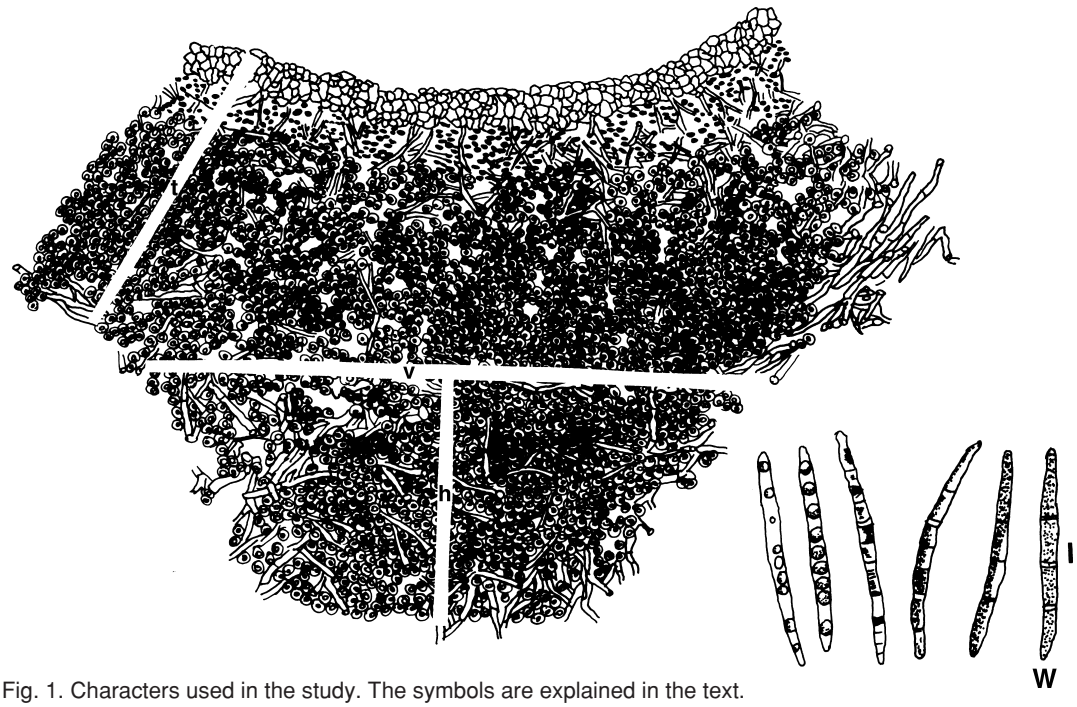


Fig. 1. Characters used in the study. The symbols are explained in the text.

MATERIAL AND METHODS

Forty-four samples were selected from different habitats, populations and altitudes in the Iberian Peninsula (Appendix 1). The material studied is deposited in MACB (herbarium of Dpto. Biología Vegetal I, Universidad Complutense, Madrid, Spain) and in personal collection of the author I. Martínez (HIMM). The taxa have been identified based on the morphology of the rhizines (densely branched and anastomosing in *Peltigera canina*, mainly squarrosely branched and isolated in *P. membranacea* and mainly simple and isolated in *P. praetextata*) and veining pattern (flattened to somewhat raised smooth veins in *P. canina*, downy and raised in *P. membranacea* and flattened and smooth in *P. praetextata*). The presence/absence of phyllidia has not been considered in the identification of *P. praetextata*. The samples with intermediate morphological characters were excluded in the study to avoid misidentifications.

Six characters were analysed in each specimen. For each sample, the following measurements were carried out (Fig. 1):

- 20 mature released spores (width (w) and length (l)) from five different apothecia,
- 20 measurements of thalli (thallus thickness between veins (t), vein width (v) and vein height (h)). From each sample five different lobes were selected and four single sections through each lobe were made. For each section one measurement was made, and the altitude of the sample origin was recorded.

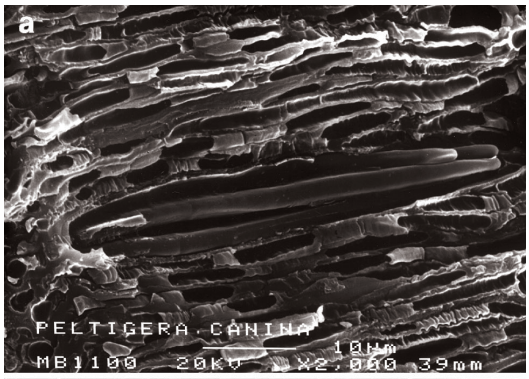
The cross-sections of thalli were made by freezing microtome from medium lobe location because at this area the thallus has its maximum thickness (Martínez & Burgaz 1994). The sections were examined in distilled water and lactophenol cotton blue. Released spores were measured from handcut sections of apothecia. The spores were considered mature when three septa appeared and no cytoplasmatic bridges or lipidic drops were observed. The spore wall ornamentation was studied with scanning electron microscopy (SEM).

Statistical analysis of the data was performed using Statistical Package Statgraphics (version 5.0) of Statistical Graphics Corporation (1987) and SPSS/PC+ (version 3.0) developed by Norusis (1986). An ANOVA analysis was used to see if the data differed significantly. The data were subjected to a logarithmic transformation (Økland 1990) to standardize the variables. Two multivariate statistical methods were carried out, a Principal Component Analysis (PCA) and Discriminant Analysis (DA).

RESULTS AND DISCUSSION

The results of the ANOVA analysis presented in Table 1 show several differences in the anatomical features of the three species, and in their altitudinal range.

Peltigera membranacea has the longest and narrowest spores, thinnest thallus and, narrowest and highest veins. By contrast, *P. praetextata* has the shortest spores and, widest and flattest veins. *Peltigera canina* has a thallus thickness and spore size



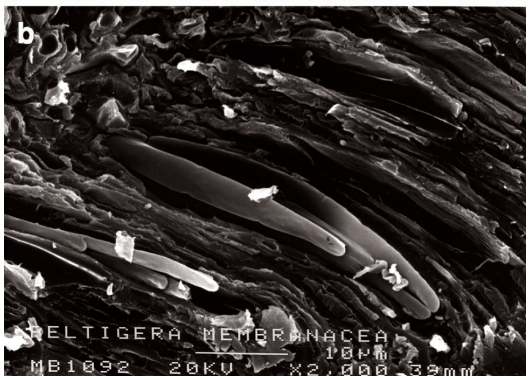


Fig. 2. SEM migrographs of spore oenamentation. — a: *Peltigera canina* (L.) Willd. — b: *P. membranacea* (Ach.) Nyl. — c: *P. praetextata* (Sommerf.) Zopf.

similar to *P. praetextata* and a veining pattern intermediate between *P. membranacea* and *P. praetextata*.

Although all the characters differed significantly as shown by the ANOVA analysis, the mean values of some individual variables in *Peltigera canina* and *P. praetextata* are very close. Comparing the mode of the data to avoid extreme values, similar results to mean values are observed between *P. canina* and *P. praetextata*. Otherwise, *P. membranacea* has less overlapping ranges with the other taxa.

The spores of the three species are acicular in shape (length/width > 5) and lack cell wall ornamentation as was observed in with SEM (Fig. 2).

Since all characters had significantly different means, only those characters which summarized higher variation of the data set in the Principal Component Analysis (PCA) were used, such as spore length, thallus thickness between veins and vein height. In Fig. 3, the first and second component summarized 58% of the variation in the data set, though a great overlapping of the data is detected within the three species, so a second PCA ordination was performed in order to try to improve the results.

In a comparison between *Peltigera canina* and *P. praetextata* (Fig. 4), only the vein height shows the split between these species (summarized 77% of the variation). The spore length and the thallus thickness between veins are useless in distinguishing them.

Table 1. Means ± standard deviations, [mode], and (ranges) of variables (in µm). *p* = significance level (*** = $p < 0.001$; ** = $p < 0.01$; * = $p < 0.05$; *ns* = not significant). Test of significance refer to ANOVA.

Variables	*P. canina*	*P. membranacea*	*P. praetextata*
Spore width	4.28 ± 0.43 [4.47] (3.20–5.33)	3.97 ± 0.43 [3.77] (2.71–5.03)	4.17 ± 0.45 [4.6] *** (2.71–5.58)
Spore length	49.68 ± 7.60 [49.5] (29.66–76.34)	53.98 ± 5.75 [53.43] (38.45–71.92)	46.53 ± 6.96 [44.74] *** (28.03–74.59)
Thallus thickness Between veins	226.42 ± 33.84 [216.4] (161.91–324.53)	184.65 ± 40.01 [183.44] (114.22–332.45)	229.27 ± 43.12 [198.15] *** (155.22–356.03)
Vein width	675.40 ± 168.22 [648.81] (430.49–1 123.32)	590.77 ± 152.10 [554.82] (371.11–1 051.07)	748.48 ± 213.96 [748.16] *** (320.25–1 283.79)
Vein height	246.31 ± 68.21 [278.1] (129.24–421.19)	259.161 ± 62.41 [254.01] (131.18–412.58)	237.66 ± 65.00 [260.87] *** (135.33–389.20)
Altitude	1 238.43 ± 325.76 [1 000] (838–2 000)	1 003 ± 378.02 [1 100] (30–1 400)	1 100.78 ± 330.13 [1 100] *** (500–1 650)

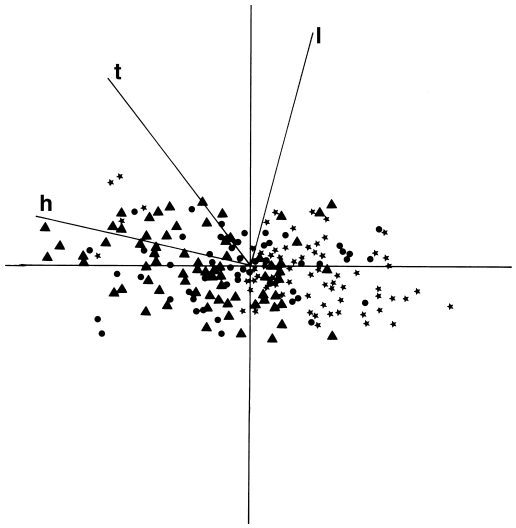


Fig. 3. Plot of the first two principal components scores (PCA) of the studied species. ● – *Peltigera canina* (L.) Willd., ★ – *P. membranacea* (Ach.) Nyl., ▲ – *P. praetextata* (Sommerf.) Zopf; l – spore length, t – thallus thickness between veins, h – vein height.

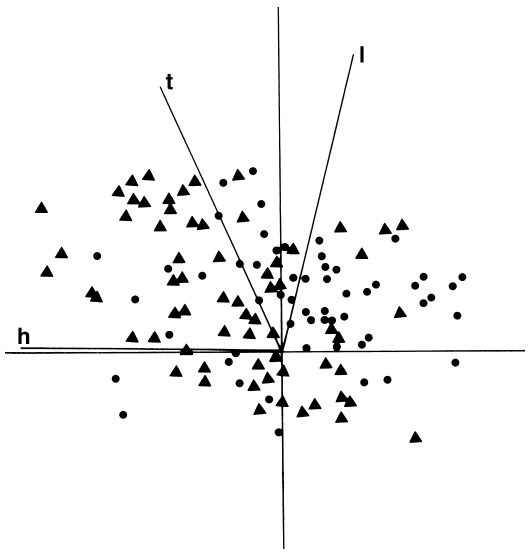


Fig. 4. Plot of the first two principal components scores (PCA) of *Peltigera canina* (L.) Willd and *P. praetextata* (Sommerf.) Zopf; ● – *P. canina*, ▲ – *P. praetextata* l – spore length, t – thallus thickness between veins, h – vein height.

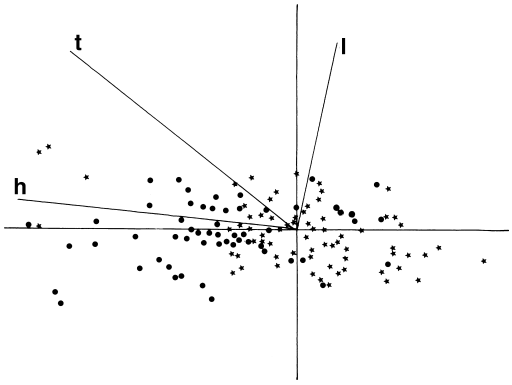


Fig. 5. Plot of the first two principal components scores (PCA) of *Peltigera canina* (L.) Willd. and *P. membranacea* (Ach.) Nyl. ● – *P. canina,* ▲ – *P. membranacea*; l – spore length, t – thallus thickness between veins, h – vein height.

The vein height is also the most useful variable to separate *Peltigera canina* and *P. membranacea*. In addition, thallus thickness between veins may be informative in separating the two taxa. This analysis summarized 79% of the variation (Fig. 5).

To compare *Peltigera membranacea* and *P. praetextata*, 87% of the variation is summarized (Fig. 6). The vein height is the most useful character here, too. In addition, the spore length and the thallus thickness between veins also tend to separate them.

After these results, *Peltigera membranacea* is the species which shows higher differentiation com-

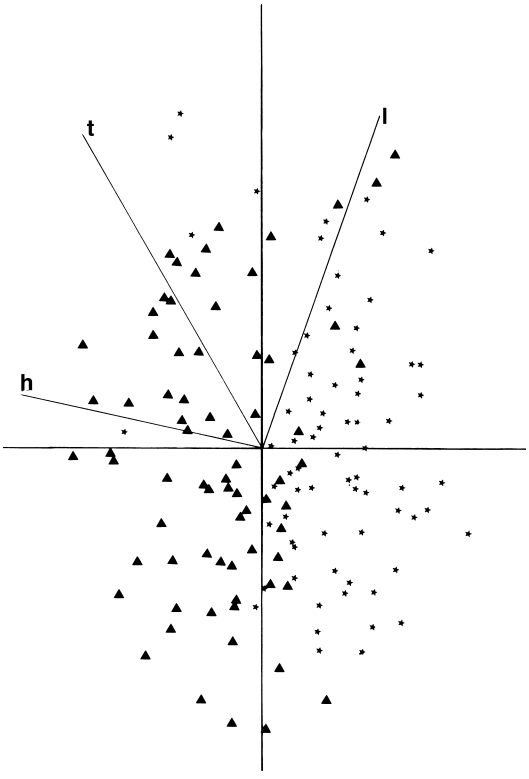


Fig. 6. Plot of the first two principal components scores (PCA) of *P. membranacea* (Ach.) Nyl. *P. praetextata.* (Sommerf.) Zopf. ★ – *P. membranacea*, ▲ – *P. praetextata,* l – spore length, t – thallus thickness between veins, h – vein height.

Table 2. Results of stepwise selection of characters. *p* = significance level. *F* = Fisher's *F*.

Step no.	Variables	*p*	*F*	Wilk's Lambda
1	Thallus thickness	***	11.7186	0.89557
2	Vein height	***	8.81633	0.84452
3	Spore width	***	7.16306	0.81458
4	Vein width	***	5.77233	0.80204
5	Spore length	***	4.85840	0.79250

Table 3. Functions effective by statistic criteria.

Function	Eigenvalue	Canonical correlation	Percentage of variance
1	0.2067	0.4139	81.92
2	0.0456	0.2089	18.08

Table 4. Results of stepwise selection of characters between *P. canina* and *P. praetextata*. *p* = significance level. *F* = Fisher's *F*.

Step no.	Variables	*p*	*F*	Wilk's Lambda
1	Vein height	**	9.05880	0.93668
2	Spore width	**	5.04166	0.92953
3	Vein width	*	3.69550	0.92252

Table 5. Functions effective by statistic criteria between *P. canina* and *P. praetextata*.

Function	Eigenvalue	Canonical correlation	Percentage of variance
1	0.0840	0.2784	100.0

Table 6. Results of stepwise selection of characters between *P. canina* and *P. membranacea*. *p* = significance level. *F* = Fisher's *F*.

Step no.	Variables	*p*	*F*	Wilk's Lambda
1	Thallus thickness	***	15.851	0.8905
2	Spore width	***	9.908	0.86594
3	Vein width	***	7.868	0.84326
4	Spore length	***	6.248	0.83448

Table 7. Functions effective by statistic criteria between *P. canina* and *P. membranacea*.

Function	Eigenvalue	Canonical correlation	Percentage of variance
1	0.1984	0.4068	100.0

Table 8. Results of stepwise selection of characters between *P. praetextata* and *P. membranacea*. *p* = significance level. *F* = Fisher's *F*.

Step no.	Variables	*p*	*F*	Wilk's Lambda
1	Thallus thickness	***	19.998	0.874
2	Spore length	***	13.6786	0.83456
3	Vein height	***	10.5897	0.81176
4	Spore width	***	9.10912	0.78870
5	Vein width	***	7.49235	0.78278

Table 9. Functions effective by statistic criteria between *P. praetextata* and *P. membranacea*.

Function	Eigenvalue	Canonical correlation	Percentage of variance
1	0.2775	0.4661	100.0

pared with the other two species (summarizing 79% and 87% of the variation), whereas *P. canina* and *P. praetextata* have more similarity (summarizing only 77% of the variation). The vein height is the best discriminating character for these three species.

In order to attempt better results a Discriminant Analysis (DA) was run. This analysis determines if, with the original variables, the taxa are sufficiently defined, and besides it is a method of selecting variables which try to find those that best reveal the differences among the groups. It was preferred to make a stepwise analysis, because in this case, only the variables with a higher discriminant power are selected. The selection of the variables was done using the "Wilk's Lambda" values, so that in each step one variable which minimizes the Wilk's Lambda is selected.

The results of the DA show a poor dissimilarity between the taxa because the Wilk's Lambda values are high and the "F" values are low (Table 2). Also, the functions validity (Table 3) prove that the success level in the identification of taxa is 53.92%.

As the effectiveness to discriminate the three species together is low, new DA analyses were run (Tables 4–9). Three characters discriminate *Peltigera canina* and *P. praetextata*, namely the vein height, spore and vein width (58% of success); while four

characters discriminate *P. canina* and *P. membranacea* with a 72% of success, namely the thallus thickness between veins, spore and vein width and spore length.

All the characters except altitude identify *Peltigera praetextata* and *P. membranacea* with a 74% of success.

The results of the DA analysis show that *Peltigera canina* and *P. praetextata* are anatomically more similar taxa, and the characters to distinguish between them are fewer than those separating them from *P. membranacea*.

CONCLUSIONS

The statistical analyses prove that the relationships between *Peltigera canina* and *P. praetextata* are closest, while *P. membranacea* is anatomically and morphologically better characterized and being more easily segregated within this group. The converging in the anatomical values of *P. canina* and *P. praetextata* coincide with the morphological variation observed because a great morphological intraspecific variation is shown by in these species. However, the final conclusion supports recognition of the entities considered in this study as three different species.

Several anatomical characters were found useful to define these species. The thallus thickness between veins is a good character to identify *Peltigera membranacea* and *P. canina* (Martínez & Burgaz 1994) and this was supported now by the PCA and DA analyses. This character is also statistically effective to identify *P. membranacea* and *P. praetextata*.

In addition, the vein height is a good character, too, in distinguishing *Peltigera praetextata* from the other two species, and is the only differential anatomical character to separate *P. canina* and *P. praetextata*.

The results received in the study of spore size concur with the data of Vitikainen (1994). The specimens of *Peltigera membranacea* have longer spores than *P. canina*, and both have longer spores than *P. praetextata*, but this tendency in the spore size is more effective to separate *P. membranacea* and *P. praetextata* than *P. membranacea* and *P. canina*.

The analyses indicate that these taxa share the same altitudinal gradient, and they usually grow in similar habitats and areas. *Peltigera praetextata* appears to be widespread in several altitudinal belts, while *P. canina* is more continental tendencies, and *P. membranacea* prefers less continental habitats.

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APPENDIX

Specimens examined

Peltigera canina (L.) Willd.

ANDORRA: Les Escaldes-Engordany, collado de Jovell, 31TCH80, 1 650 m, *Herrero*, HIMM 342. **SPAIN:** ASTURIAS: Somiedo, Villar de Vildas, 29TQH1772, 1 000 m, *Martínez et al*., HIMM 196. BURGOS: Sta. Cruz del Valle Urbión, sierra de la Demanda, 30TVM88, 1 100 m, *Martínez et al*., MACB 44606. CÁCERES: Garganta la Olla, arroyo Majaelbrera, 30TTK6043, 1 100 m, *Aragón & Castillo*, HIMM 7. CIUDAD REAL: puerto de Valderepisa, sierra Madrona, 30SUH8159, 838 m, *Burgaz*, HIMM 463 (2). GUADALAJARA: Torremocha del Campo, La Fuensaviñán, 30TWL3533, 1 000 m, HIMM 19. JAÉN: Beas de Segura, sierra de las Cuatro Villas, 30SWH1330, 1 200 m, *Pajarón et al*., HIMM 109. LEÓN: Villablino, Rioscuro, 30TQH2256, 1 450 m, *Burgaz & Martínez*, HIMM 277. LÉRIDA: Espot, riu Monestero, 31TCH3616, 2 000 m, *Martínez et al*., HIMM 163. MADRID: Cadalso de los Vidrios, barranco del Boquerón, 30TUK7964, 850 m, *Aragón & Castillo*, MACB 48574. La Pedriza, cuenca alta del Manzanares, 30TVL21, 1 130 m, *Herrero*, HIMM 319. Lozoya, puerto de Navafría, 30TVL33, 1 400 m, *Burgaz*, HIMM 116. SEGOVIA: Cedillo de la Torre, Umbría de la Hoz, 30TVL4686, 1 000 m, Martínez *et al*., HIMM 15. TERUEL: valle del Tajo, sierra de Albarracín, 30TXK18, 1 600 m, *Ibáñez*, HIMM 17.

Peltigera membranacea (Ach.) Nyl.

PORTUGAL: BEIRA ALTA: sierra de la Estrella, 29TPE27, 1 100 m, *Burgaz & Martínez*, HIMM 429. BEIRA LITORAL: Luso, sierra de Bussaco, 29TNE56, 500 m, *Burgaz & Martínez*, HIMM 426. **SPAIN:** ÁLAVA: Caicedo Yuso, lago de Arrero, 30TWN0036, 660 m, *Herrero*, HIMM 323. ASTURIAS: Somiedo, Valle de Lago, 29TQH3072, 1 400 m, *Martínez et al*., HIMM 70. BURGOS: Santa Cruz del Valle Urbión, sierra de la Demanda, 30TVM88, 1 100 m, *Burgaz*, MACB 48575. Huidobro, 30TVN43, 850 m, *Burgaz*, HIMM 363. CANTABRIA: Comunidad de Campoo de Cabuérniga, río Saja, 30TUN9472, 850 m, *Martínez et al*., MACB 56228. LA CORUÑA: Betanzos, río Mandeo, 29TNH6691, 30 m, *Burgaz & Martínez*, HIMM 278 (2). HUESCA: Hecho, 30TXN8744, 1 200 m, *Izquierdo*, HIMM 644. LÉRIDA: Las Bordas, valle de Artiga de Lin, 31TCH12, 1 200 m, *Aragón & Martínez*, HIMM 72. MADRID: Canencia, arroyo del Sestil del Mahillo, 30TVL3426, 1 300 m, *Aragón & Martínez*, HIMM 738. MÁLAGA: Estepona, sierra Bermeja, los Reales, 30SUF0240, 1 350 m, *Aragón & Martínez*, HIMM 509. SEGOVIA: Riofrío de Riaza, valle del río Riaza, 30TVL6364, 1 390 m, *Martínez et al*., HIMM 386. ZAMORA: Sanabria, 29TPG86, 1 100 m, *Burgaz*, MACB 34864.

Peltigera praetextata (Sommerf.) Zopf

FRANCE: Pyrenées, arroyo de Arreno, lagos de Remoulis, 30TYN24, 1 650 m, *Herrero*, HIMM 123. **PORTUGAL:** BEIRA ALTA: sierra de la Estrella, 29TPE27, 1 100 m, *Burgaz & Martínez*, HIMM 847. BEIRA LITORAL: Luso, Bussaco, 29TNE56, 500 m, *Burgaz & Martínez*, HIMM 122. **SPAIN:** ASTURIAS: Somiedo, río Pigüeña, Brañas de los Cuartos, 29TQH1971, 1 240 m, *Martínez et al*., HIMM 91. ÁVILA: Candeleda, arroyo de Santa María, 30TUK1053, 700 m, *Martínez et al*., MACB 48865. BURGOS: Santa Cruz del Valle Urbión, sierra de la Demanda, 30TVM88, 1 100 m, *Martínez et al*., MACB 44586. CANTABRIA: Camaleño, Invernales de Mato, 30TUN5574, 950 m, *Burgaz*, HIMM 239. CIUDAD REAL: Fuencaliente, río Cereceda, 30SUH9256, Sarrión, MACB 43892. JAÉN: sierra de Segura, las Acebeas, 30SWH3741, 1 200 m, *Herrero*, MACB 44584. LÉRIDA: Las Bordas, valle de Artiga de Lin, 31TCH12, 1 200 m, *Aragón & Martínez*, HIMM 115. LUGO: Castroverde, Cubelas, 29TPH3764, 780 m, *Munín*, MACB 48875. NAVARRA: Mugaire-Oronoz, puerto Otxondo, 30TXN28, 620 m, *Ahti & Burgaz*, MACB 44591. LA RIOJA: puerto de Piqueras, Lumbreras, 30TWM35, 1 560 m, *Burgaz & Mendiola*, MACB 14678. SEGOVIA: Riofrío de Riaza, hayedo de la Pedrosa, 30TVL6563, 1 605 m, *Munín & Rojas*, HIMM 118. TOLEDO: Las Navillas, monte del Cuervo, 30SUJ87, 1 100 m, *Martínez*, MACB 44592. ZARAGOZA: Tarazona, sierra de Moncayo, barranco de Castilla, 30TXM02, 1 300 m, *Martínez*, HIMM 120.