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Myxomycetes (Amoebozoa) occurring on *Cordyline australis* (G. Forst.) Endl.

Óscar Requejo[1] and N. Floro Andrés-Rodríguez[1]

[1] Galician Mycological Group "Luís Freire". Marqués de Valladares, 14, 5º-6, 36209 Vigo-Galicia (Spain)

E-mail: oscarequejo@hotmail.com

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Abstract: This paper presents the first outcome of a study carried out in a plot with mature specimens of *Cordyline australis* (G. Forst.) Endl. The study area and methodology are described, and 18 species are identified after reviewing 41 exsiccatae, one of which we believe to be the first record to date for Galicia and another one to be the first one for Pontevedra.

Keywords: Amoebozoa, biodiversity, ecology, eumycetozoa, Galicia (Spain), phenology, slime molds.

Introduction

Cordyline australis (G. Forst.) Endl., popularly known as dracaena or cordyline, is an arboreal monocot belonging to the family Asparagaceae Juss. that is native to New Zealand and has been introduced worldwide as an ornamental tree. A high-quality textile fiber can be obtained from the leaves of this plant (López-González 2010), and these fibers are used to make ropes due to the great resistance they offer (Blanco-Dios and Castro 2010).

Cordyline australis trees are typically 4 to 6 m in height (Fig. 1) and can reach up to 15 (20) m (López-González 2010), with a trunk diameter of up to 1.5 m and leaves grouped in tufts at the apices of the branches. The leaves are bright green, long, tapered and arched, with dimensions of up to 100 x 6 cm. Plants bloom in late spring or early summer, producing inflorescences in clusters formed by groups of small (5-6 mm) aromatic flowers. The inflorescences are white and have six stamens, superior ovaries with trilobed stigma termination. The plants produce fructose in the form of small white berries that are grouped in clusters.

The study of the myxomycetes in Galicia is recent (Andrés-Rodríguez and Requejo 2021) and, although the province of Pontevedra is the one with the largest number of specimens contributes to the Galician catalogue (Requejo and Andrés-Rodríguez 2022), no studies have been carried out in the Baixo Miño region, which borders with Portugal and is close to the Miño river mouth in the Atlantic Ocean. Its geographical position conditions a mixed, oceanic climate with Mediterranean influence, with mild temperatures both in winter and summer, moderate rainfall in winter and high relative humidity throughout the year.

These characteristics, along with the climatic conditions of the zone, make these trees an interesting substrate for the occurrence of myxomycetes (Lado and Rojas 2020). In addition, studies on the occurrence of myxomycetes in monospecific tree plantations are not frequent, and there are no studies analyzing the myxomycete flora associated with *C. australis*. For these reasons, this study aimed to deepen the study of these monoculture plots by periodic fresh sampling, together with the collection of material for moist chamber cultures.

Figure 1. Specimens of *Cordyline australis*.

Materials and methods

Study area

The municipality of Tomiño (Pontevedra, Galicia, Spain) is located in the southern part of the province, separated from Portugal by the Miño River. The climate of the area is classified as Csb (temperate Mediterranean with mild summers) according to the Köppen and Geiger classifications (Climate-Data.org 2022, online). Rain falls mainly in winter, with relatively low rainfall intensity in summer. The average annual temperature is 13.5 °C, and the total annual precipitation reaches 1678 mm. The variation in precipitation between the driest and wettest months is 197 mm. The mean monthly temperature is 11.2°.

The following elements (Table 1 and Fig. 2) show the complete bioclimatic data (temperature, rain, sunlight hours) for the region (adapted from Climate-Data 2022, online).

Table 1. Climatic data for Tomiño (average of 1991-2021).

	Monthly average												
	Jan	**Feb**	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**	**Annual average and totals**
Tª average (ºC)	8.3	8.5	10.4	12.0	14.4	17.5	19.2	19.5	18.0	15.0	10.8	8.9	13.5
Tª max. average (°C)	11.7	12.4	14.4	15.8	18.2	21.3	23.1	23.7	22.3	18.8	14.2	12.5	17.4
Tª min. average (°C)	5.1	4.8	6.5	8.0	10.4	13.4	15.1	15.3	14	11.5	7.8	5.7	9.8
Rain (mm)	213	145	153	152	128	57	34	44	88	224	231	209	1678
Humidity (%)	83%	80%	78%	79%	79%	78%	75%	74%	76%	81%	82%	82%	79%
Days with rain	10	8	8	10	10	6	4	4	6	10	11	10	97
Sunlight hours	4.8	5.9	6.8	8.0	8.9	9.6	9.7	9.4	8.5	6.7	5.6	4.9	7.4

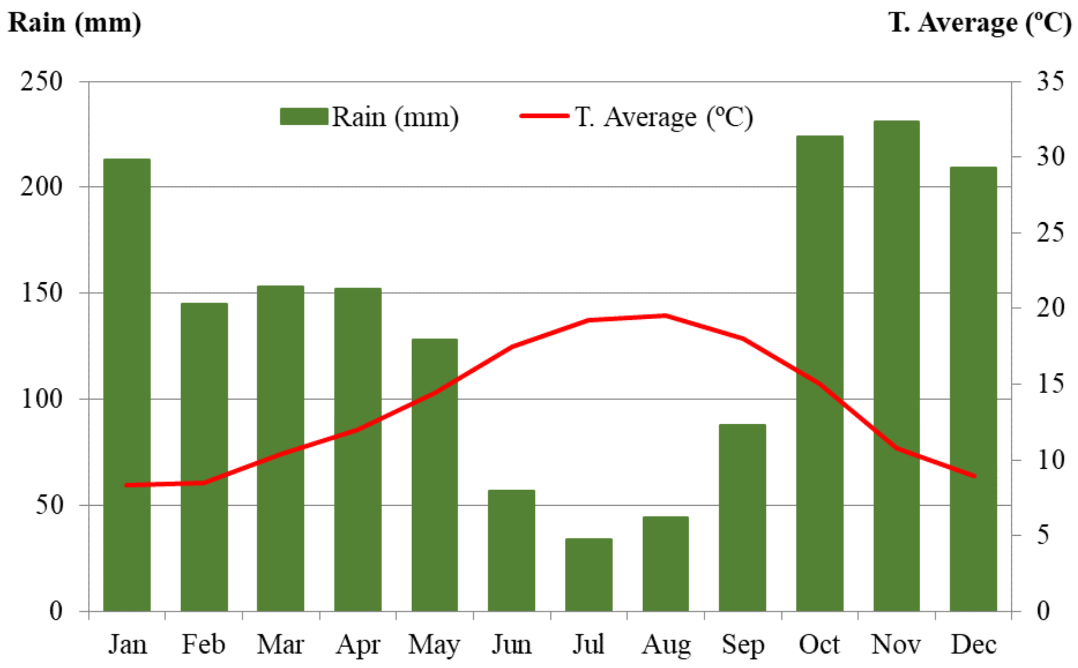

Figure 2. Climograph of Tomiño (mean from 1991-2021).

Sampling was carried out on nine dates throughout 2019 (18 April, 3 and 18 May, 12 August, 13 and 24 November) and 2020 (10 and 20 February, 10 March) in two plots with plantations of mature specimens of *C. australis*; the plantations were located close to each other in Figueiró, municipality of Tomiño, in the southern part of the province of Pontevedra, Baixo Miño region (41° 57′ 37.8″ N, 08° 45 ′

15.9 ″ O) (Fig. 3). The sampling area is located 135 m from the bed of the Miño River and at a height of 12 m above sea level and 7 m above the river. The dimensions of the two plots are as follows:

Plot 1 (41º 57’ 37.3” N, 08º 45’ 16.3” W) is shaped like a triangle, with a base of 24 m, height of 50 m, and an area of 600 m^2, and contains approximately 150 trees. The orientation of this plot runs approximately west-east.

Plot 2 (41º 57’ 39.1” N, 08º 45’ 14.3” W) is rectangular in shape, with a length of 65 m, width of 8 m, and an area of 520 m^2, and contains approximately 130 trees. The orientation of this plot runs approximately north-south.

Figure 3. Location of the study area (adapted from Google Maps 2022, online).

Both plots were first planted with *C. australis* approximately twenty years ago, when the area was used as a nursery for ornamental garden and park trees. The first seedlings came from Barcelona and Valencia. Throughout most of the area, the trees are planted in irregular frames 1.5 to 2 m in diameter (Figs. 4a and 4b), which allow little sunlight to reach the interior of the shrubs (Fig. 4c), and the understorey has a layer of fallen leaves that reaches more than 25 cm in thickness in some areas (Fig. 4d). The curves of the dry leaves accumulate small amounts of water, contributing to the environmental moisture.

Data on the average humidity throughout the year and the proximity of the sampling area to the Miño River indicate high water contents in the area; together with the characteristics of the wood and the vegetative elements of *Cordyline*, which are rich in carbohydrates, mucilage and fibre, and the range of temperatures in the area, these conditions favour the growth of bacteria, fungi, yeasts and protozoa while slowing the decomposition of plant remains.

Figure 4. Study area: a) Plot 1; b) Plot 2; c) view from inside the plantation; d) understory.

Methodology

The methodology followed the same approach typically used in the study of myxomycetes (e.g., Wrigley de Basanta and Estrada Torres 2017; Lado and Rojas 2020). Material was collected during the periodic examination of the trees and their existing remains in two plots, noting and distinguishing the plot of origin, the different elements from which the material was collected (from living and dead leaves, from clusters of flowers that had fallen and dried at the time of sampling, from the bark and wood of the trunk and from the bark of the dead fallen branches) and the state of the tree (alive or dead and standing or on the ground). The collection of fresh specimens was accompanied by the taking of samples for the laboratory. Of each sample, one part was used for cultures and another for pH determination.

Eighteen cultures were made in a moist chamber after collecting samples of these elements from *Cordyline* plants. For the collection of the samples and their culturing in the moist chambers, the methodologies described by Stephenson and Stempen (1994), Keller et al. (2008), Goad and Stephenson (2013) and Wrigley de Basanta and Estrada Torres (2017) were used.

The pH levels of the different tree structures were measured following the methodology described by Rüdiger (1975) and Poblete and Roffael (2004) with adaptations for our study. The material was crushed in the laboratory and divided (M1 and M2); the pH was determined separately for the two groups and the arithmetic mean of the two measurements was calculated to increase the precision of the results. A total of 5 g of the crushed material of each sample was added to 50 ml of deionized water (10% W/V) in glass beakers. Two pH measurements were taken (at 2 hours and 24 hours after the solutions were made), and the temperature in the laboratory was 22 °C. A Hanna Instruments H198103 pH meter was used, calibrated with buffer solutions with pH values of 4.0 and 7.0.

For the identification of the species obtained both from the samplings and in the moist chambers (Fig. 5), macroscopic observations that could not be made visually were made with a Nikon® SMZ745T stereoscopic microscope. A Nikon® Eclipse E100 microscope with a Jenoptic® CT3 digital camera was used. The microphotographs and measurements of the most relevant structures were made using the ProgRes® CapturePro 2.7 program. The samples were mounted with water or Hoyer's medium.

The literature consulted for the identification of the species was mainly Martin and Alexopoulos (1969), Nannenga-Bremekamp (1991), Neubert et al. (1993, 1995, 2000), Poulain et al. (2011) and Lado and Rojas (2020). Updated names were obtained from Web Eumycetozoa (Lado, 2005-2022), and the systematic approach is the one recently proposed by Leontyev et al. (2019).

All the samples are preserved in the herbarium of the Galician Mycological Group (GMG-myxo).

Figure 5. Moist chambers corresponding to Parcel 1.

Results

The results of two years of sampling in the two plots described include 41 specimens (30 in plot 1, and 11 in plot 2) belonging to 11 genera and 18 species. Of these taxa, we believe that one species, *H. pardina* (Minakata) Ing, is recorded for the first time in Galicia, while *P. chrysosperma* (Curr.) Lister is recorded for the first time in the province of Pontevedra.

Species catalogue

The list of species found is presented below in alphabetical order to facilitate consultation. Along with the protologue, the plot number, substrate, date, and herbarium number of the exsiccata are included. In cases where fruiting occurred in the moist chamber, the initials CH were added, and the date recorded was that of the sampling. The taxonomic classification *sensu* Leontyev et al. (2019) is provided below the list of species.

Arcyria cinerea (Bull.) Pers., *Syn. meth. fung*. 1: 184. 1801

Material studied: Plot 1, on fallen leaves, 13-XI-2019, GMG-myxo 473 CH. Plot 2, on fallen leaves, 24-XI-2019, GMG-myxo 477 CH. Ibid, on woody remains of flower clusters, 24-XI-2019, GMG-myxo 480 CH. Idem, GMG-myxo 481 CH.

Arcyria denudata (L.) Wettst., *Verh. Zool.-Bot. Ges. Wien* 35:535. 1886

Material studied: Plot 1, on wood in a debarked trunk, 10-II-2020, GMG-myxo 444.

Arcyria obvelata (Oeder) Onsberg, *Mycologia* 70(6):1286. 1979

Material studied: Plot 1, on debarked trunk, in the outer layer, 3-V-2019, GMG-myxo E202.

Craterium minutum (Leers) Fr., *Syst. mycol*. 3(1):151. 1829

Material studied: Plot 1, on dry leaves, 10-II-2020, GMG-myxo 389. Ibid, 10-III-2020, GMG-myxo 401.

Didymium difforme (Pers.) Grey, *Nat. arr. Brit. pl.* 1:571. 1821

Material studied: Plot 1, the inner part of bark, 3-V-2019, GMG-myxo E215.

Didymium melanospermum (Pers.) T. Macbr., *N. Amer. Slime-moulds,* ed. 1, 88. 1899

Material studied: Plot 1, on bark, 18-V-2019, GMG-myxo E216.

Didymium nigripes (Link) Fr., *Syst. mycol.* 3(1):119. 1829

Material studied: Plot 1, on the pedicel of a dry leaf, 3-V-2019, GMG-myxo E212. Ibid, on dried leaves, GMG-myxo E214. Ibid, on the bark of a felled tree, 13-XI-2019, GMG-myxo 475 CH. Ibid, on dead leaves on the ground, GMG-myxo 474 CH. Idem, GMG-myxo 476 CH. Plot 2, on dried leaves, 24-XI-2019, GMG-myxo 479 CH.

Didymium verrucisporum A.L. Welden, *Mycologia* 46(1):98. 1954

Material studied: Plot 1, on dry leaves, 20-II-2020, GMG-myxo 395.

Hemitrichia calyculata (Speg.) M.L. Farr, *Mycologia* 66(5):887. 1974

Material studied: Plot 1, on wood from a trunk, 10-III-2020, GMG-myxo 445.

Hemitrichia pardina (Minakata) Ing, *Myxomycetes of Britain and Ireland*, 132. 1999

Material studied: Plot 1, on bark, 13-XI-2019, GMG-myxo 466 CH. Ibid, on the bark of a branch on the ground, 13-XI-2019, GMG-myxo 482 CH (Fig. 6).

Description: Sporocarpic fructifications, solitary or scattered, with 0.4-1 mm total height. Stipe of 0.2-0.6 mm, cylindrical with slightly helical grooves, blackish brown, reddish brown in transmitted light. Sporotheca globose to subglobose, up to 0.7 mm in diameter, yellow or ochre yellow, ornamented with thick clumps of amorphous material dispersed on the surface as prominent warts. Double peridium, membranous inner layer, and gelatinous outer layer. Capillitium formed by tangled tubules of 2-4 (5) µm in diameter, covered with dark spines up to 2.5 µm in length and with faint spiral relief; barely have free ends, and if they have them, the tips are short and pointed. The capillitium has thickenings of (7) 9-12 (25) x (4) 5-7 (18) µm. Spores in mass yellow, creamy yellow, with a certain greenish hue under transmitted light, 9-11 µm in diameter and covered with fine spines.

In the literature, the sporotheca is described as large relative to the stalk, although in our specimens, the length of the sporotheca is equal to or greater than its diameter, which may be due to the growth of specimens in the moist chamber under conditions other than those found in the natural environment.

Despite its small size, which could cause it to go unnoticed, *H. pardina* is well known throughout the American and European continents. In the Iberian Peninsula, its distribution is centred on the Mediterranean coast, but we did not find any record of this species in Galicia.

Hemitriahia pardina is a relatively easy species to identify due to its small size and the prominent dark brown warts scattered on the surface of the sporotheca. *Hemitrichia minor* G. Lister is a similar species, of which *H. pardina* was considered a variety in the past (Nannenga-Bremekamp, 1991, Neubert et al, 1993), but today is accepted as independent species (Ing, 1999, Poulain et al, 2011). *H. minor* has slightly smaller sporocarps, up to 0.6 mm, and the spores are larger (10-13 µm in diameter versus 9-11 µm for *H. pardina*) (Poulain et al., 2011).

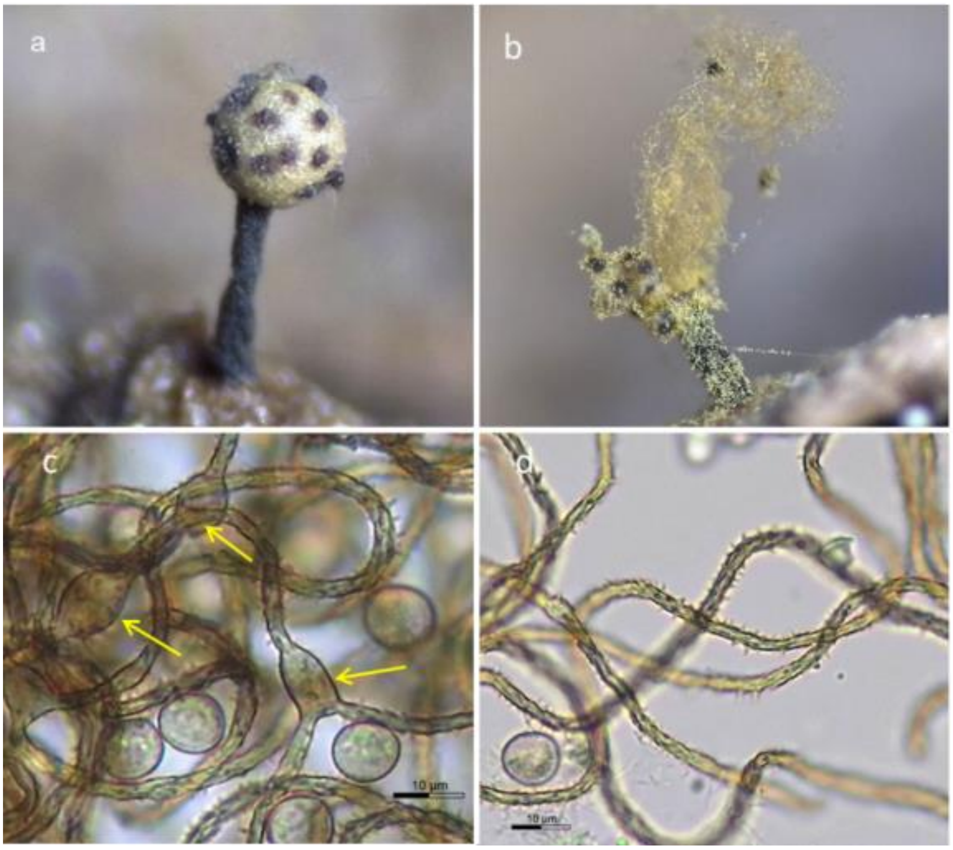

Figure 6. *Hemitrichia pardina*. a) Sporocarp. b) Mature sporocarp. c) Spores and capillitium (thickenings are indicated). d) Detail of the capillitial filaments with spines.

Leocarpus fragilis (Dicks.) Rostaf., *Sluzowce monogr*. 132. 1874

Material studied: Plot 2, on dry leaves, 24-XI-2019, GMG-myxo E256. Ibid, on a leaf pedicel, 10-III-2020, GMG-myxo 398. Ibid, on bark, 10-III-2020, GMG-myxo 399. Ibid, on dried leaves, 10-III-2020, GMG-myxo 400.

Metatrichia floriformis (Schwein.) Nann.-Bremek., *Proc. Kon. Ned. Akad. Wetensch., C*. 88(1):127. 1985

Material studied: Plot 1, on the wood of a fallen trunk, 10-II-2020, GMG-myxo 390. Plot 2, on the bark of a fallen trunk, 10-III-2020, GMG-myxo 403.

Mucilago crustacea P. Micheli ex F.H. Wigg., *Prim. fl. holsat*. 112. 1780

Material studied: Plot 1, on a living tree trunk and fallen leaves, 18-IV-2019, GMG-myxo E224. Ibid, on the pedicel of a leaf, 18-IV-2019, GMG-myxo E251. Idem, 12-VIII-2019, GMG-myxo E254. Idem, 13-XI-2019, GMG-myxo E252. Ibid, on live tree bark, 13-XI-2019, GMG-myxo E253.

Observations: *Mucilago crustacea* is a very common species in the study area. Fruiting on the trunk of a living tree showed a curious pattern, growing transversely 25-30 cm from the ground, and this pattern was observed on several trees at the same time (Fig. 7).

Figure 7. Fruiting of *Mucilago crustacea* showing a horizontal growth pattern.

Perichaena chrysosperma (Curr.) Lister, *Monogr. mycetozoa*, ed. 1, 196. 1894

Material studied: Plot 2, on leaves on the ground, 24-XI-2019, GMG-myxo 476 CH (Fig. 8).

Description: Fructifications in form of sessile sporocarps or, generally, as short plasmodiocarps in the shape of sausages or rings, 1-3 mm in length and 0.4-0.8 mm in diameter. Double peridium of yellowish-brown colour, red brown on the outside and yellow on the inside, which when ripening opens irregularly although with a circumcised tendency. Capillitium light yellow or gold, with thorns and warts. Yellow spore mass, light yellow in transmitted light, globose, 8-11 µm in diameter, finely warty.

Perichaena chrysosperma is not uncommon in the Iberian Peninsula, although in Galicia, until now, it had only been found in the province of A Coruña; therefore, this is the first record of this species in Pontevedra.

Figure 8. *Perichaena chrysosperma*. a) Immature plasmodiocarp. b) Mature plasmodiocarp. c) Spores and capillitium

Physarum bivalve Pers., *Ann. Bot. (Usteri)* 15:5. 1795

Material studied: Plot 1, on a leaf pedicel, 20-II-2020, GMG-myxo 396.

Physarum pusillum (Berk. & M.A. Curtis) G. Lister, in Lister, *Monogr. mycetozoa, ed.* 2, 64. 1911

Material studied: Plot 1, on bark, 3-V-2019, GMG-myxo E200. Ibid, on wood, in the inner weft of the trunk, 3-V-2019, GMG-myxo E201. Ibid, on a trunk, in a superficial weft, 3-V-2019, GMG-myxo E203. Ibid, on leaves, 12-VIII-2019, GMG-myxo 404. Idem, 24-XI-2019, GMG-myxo 386. Idem, 20-II-2020, GMG-myxo 394.

Stemonitopsis typhina (F.H. Wigg.) Nann.-Bremek., *Nederlandse Myxomyceten (Zutphen)* 209.1975

Material studied: Plot 2, on a felled trunk, 13-XI-2019, GMG-myxo E257.

Trichia varia (Pers. ex J.F. Gmel.) Pers., *Neues Mag. Bot.* 1:90. 1794

Material studied: Plot 1, on a trunk, 24-XI-2019, GMG-myxo 387.

Taxonomic classification of the mentioned species

Class MYXOMYCETES G. Winter, Rabenh.

Subclass LUCISPOROMYCETYDAE Leontyev, Schnittler, S.L. Stephenson, Novozhilov & Shchepin, *subcl. nov*

Order Trichiales T. Macbr

Family Trichiaceae Chevall.

Hemitrichia calyculata (Speg.) M.L. Farr,
Hemitrichia pardina (Minakata) Ing
Arcyria cinerea (Bull.) Pers.
Arcyria denudata (L.) Wettst.
Arcyria obvelata (Oeder) Onsberg,
Perichaena chrysosperma (Curr.) Lister
Trichia varia (Pers. ex J.F. Gmel.) Pers.
Metatrichia floriformis (Schwein.) Nann.-Bremek.

Subclass COLUMELLOMYCETIDAE Leontyev, Schnittler, S.L. Stephenson, Novozhilov & Shchepin, *subcl. nov*

Order Stemonitidales T. Macbr.

Family Amaurochaetaceae Rostaf. ex Cooke

Stemonitopsis typhina (F.H. Wigg.) Nann.-Bremek.

Order Physarales T. Macbr

Family Didymiaceae Rostaf. ex Cooke

Didymium difforme (Pers.) Gray
Didymium melanospermum (Pers.) T.Macbr.
Didymium nigripes (Link) Fr.
Didymium verrucisporum A.L.Welden
Mucilago crustacea F.H. Wigg., Prim.

Family Physaraceae Chevall.

Craterium minutum (Leers) Fr.
Leocarpus fragilis (Dicks.) Rostaf.
Physarum bivalve Pers.
Physarum pusillum (Berk et M.A.Curtis) G.Lister

The number of moist chambers prepared with samples collected from the various substrate structures to attempt the growth and fruiting of myxomycetes was 18, of which 8 (44,4%) produced fructifications, corresponding to 11 specimens belonging to 4 species.

The distribution of the number of specimens found throughout the year and the monthly number of samples is shown in Fig. 9.

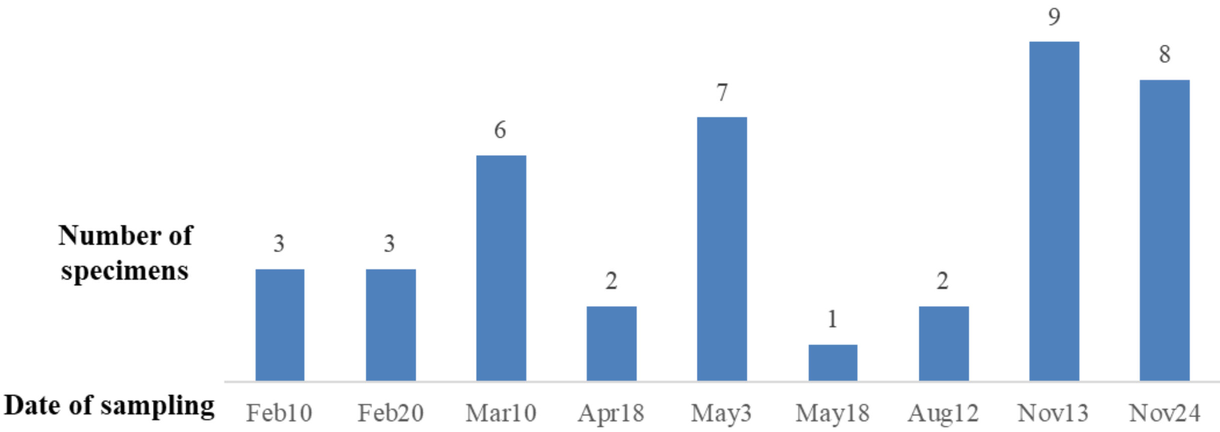

Figure 9. Monthly distribution of the specimens found.

The average of exsiccatae by sampling is 4.6±3.0 (1-9). The number of exsiccatae obtained in each sampling is shown in Fig. 10.

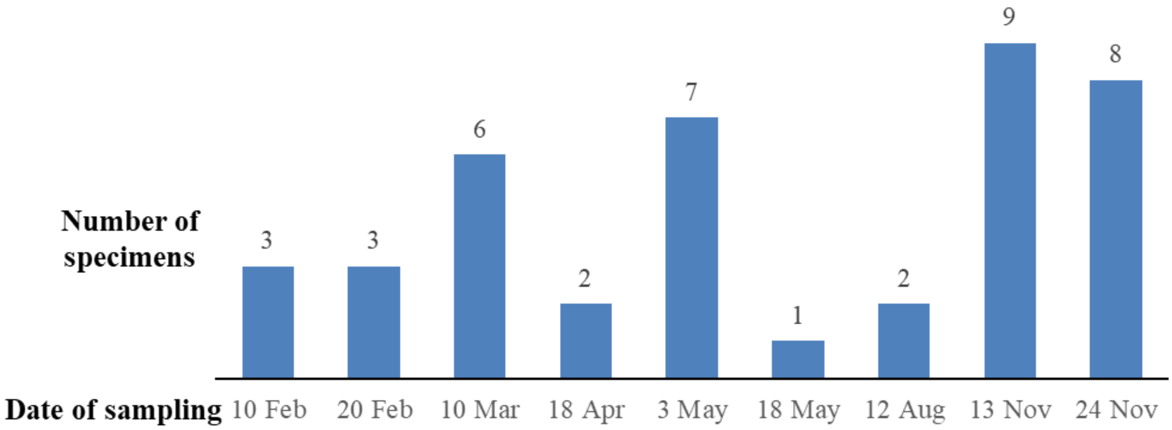

Figure 10. Number of specimens from each sampling.

The pH values of the different structures of the trees that generally serve as substrates for the growth of the myxomycetes were determined. No significant difference was found between the mean pH values of each structure between the determinations at 2 h and 24 h (t-Student: 0.1928; p-value: 0.8510).

Discussion

Although at the moment the number of exsiccatae studied (41) is not sufficient to make categorical statements, the results obtained seem to show a greater presence of specimens of the order Physarales T. Macbr., 27 (74.2%), with five genera and nine species, with the most frequently observed species being *P. pusillum* and *D. nigripes*, with six exsiccatae each, and *M. crustacea* with five exsiccatae. Greater numbers of specimens were found in spring (10), autumn (17) and winter (12) than in summer (2). This finding is consistent with the greater number of visits conducted in these seasons; however, it seems that species belonging to the Physarales order have a certain influence on the phenological pattern of fructifications, consistent with the results found in Requejo and Andrés-Rodríguez (2022, *in press*).

Table 2. Results of pH measurement of *Cordyline australis* structures.

Substrate	pH 2 h		pH 24 h	
	M1	M2	M1	M2
Fresh wood fibers from the tree	4.7	4.7	5.0	4.9
Bark of the living tree	5.3	5.4	5.9	5.8
Clusters of dried flowers	5.3	5.3	5.5	5.4
Bark from dead branches on the ground	8.4	8.3	7.6	7.5
Dry leaves fallen on the ground	6.5	6.5	5.9	5.7
Green leaves on the tree	5.6	5.6	5.4	5.4

A large variation (SD = 3.0) is observed in the number of specimens obtained in each sampling. It may be due to the environmental conditions at the time of the visit, more favorable in autumn or early spring than in the warmer months, which conditions the state of the life cycle in which the myxomycetes present in the substrates are located. Changes can be important in short periods of time, as seen in the different results of the two visits in May. All this suggests the phenological pattern described, which we consider provisional at the moment.

Although the number of specimens found in plot 1 is much higher than in plot 2, the small sample size does not allow us to establish comparisons with statistical value. For the same reason we do not dare to advance an explanation, because the proximity between the two plots would make us expect similar results. We point out, however, the plot of origin of the exsiccatae in order to be able to check in the future if these differences are maintained and we can find some differential factor.

The pH values (Table 2) indicate that most of the structures tested have an acidic character, with the exception of the bark of dead parts of the tree, which seem to have more alkaline pH, with values slightly higher than 7. In the absence of more data, we observed that the bark was more appropriate than other surfaces for the fruiting of Physarales. This affinity was already indicated by Novozhilov et al. (2017), as was the acidophilic tendency of Stemonitales, Liceales, and Trichiales, which was also evident in this study. We believe that the absence of differences between the pH measurements at 2 and 24 h suggests, despite their limited number, a certain temporal stability that confirms the accuracy of the results. From these it also seems to be deduced that, with the exception of the clusters of dried flowers, fresh substrates have higher acidity than dry ones, unlike what was found by Rüdiger (1975), which makes it necessary to have a greater number of determinations.

The pH levels of the structures of the tree that generally serve as substrates for myxomycetes, together with the water retention capacity, temperature and humidity, seem to indicate that the environment is very suitable for the growth and fruiting of Myxomycetes, which is evidenced by the high number of taxa found. Therefore, we hope to continue the study of this interesting area in the coming years through new systematic samplings that will expand upon and provide specific details about the results obtained thus far.

Acknowledgements

We thank the owners of Viveros Río Nilo, to whom the studied plots belong, for allowing us to use their facilities to carry out our study and for the information about the characteristics of the *C. australis* plantation. We also thank all the members of the Galician Mycologic Group for their company and help with the sampling and transfer of material for its registration and study, with a special mention to Mauro Rivas Ferreiro for his help with the translation of the texts.

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