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Matteo Bargagliotti [1], Daniele Canepa [2], Riccardo Vaira [3]

STUDY ON THE POPULATION OF *LYCOPODIELLA INUNDATA* (L.) HOLUB - AGORAIE DI SOPRA E MOGGETTO ORIENTED AND BIOGENETIC NATURE RESERVE (LIGURIA, NORTHERN ITALY)

Abstract - M. Bargagliotti, D. Canepa, R. Vaira, *Study on the population of* Lycopodiella inundata *(L.) Holub - Agoraie di Sopra e Moggetto Oriented and Biogenetic Nature Reserve (Liguria, northern Italy).*

This study investigated the population of Lycopodiella inundata (L.) Holub in the Agoraie di Sopra e Moggetto Oriented and Biogenetic Nature Reserve in Liguria, Italy. The population represents a significant floristic presence in the Italian Peninsula, where the species exhibits a relict and fragmented distribution. The covered area, number of individuals, density and distribution of the population were examined in 2023, with plans to continue monitoring in 2024 and 2025. This study aimed to gather sufficient data for the Population Viability Analysis (PVA). Monitoring was conducted using a grid system with 1 m² squares, further subdivided into quadrants. Individual ramets were counted and categorized as prostrate (vegetative) or ascending (reproductive) ramets. In 2023, 1159 ramets were recorded, comprising 949 vegetative individuals and 210 reproductive individuals. The covered area was approximately 18.75 m², with L. inundata documented in 75 quadrants. The distribution was confined to a narrow band parallel to the shore of Lake Riondo, with higher densities observed in areas with reduced ground cover by other species and around the cavities. The critically endangered (CR) conservation status of L. inundata at the regional level was confirmed because of the limited number of reproductive individuals. The protection and monitoring provided by the Carabinieri Forestali in the reserve has been effective in conserving the species by preventing indiscriminate collection, grazing, and excessive trampling.

Key words - *Lycopodiella inundata*, Covered Area, density, distribution, population, Agoraie

Riassunto - M. Bargagliotti, D. Canepa, R. Vaira, *Studio sulla popolazione di* Lycopodiella inundata *(L.) Holub - Riserva naturale orientata e biogenetica Agoraie di Sopra e Moggetto (Liguria).*

Questo studio ha esaminato la popolazione di *Lycopodiella inundata* (L.) Holub nella Riserva Naturale Orientata e Biogenetica delle Agoraie di Sopra e Moggetto in Liguria, Italia. La superficie coperta, il numero di individui, la densità e la distribuzione sono stati esaminati nel 2023, con l'intenzione di continuare il monitoraggio nel 2024 e nel 2025. Questo studio mira a raccogliere dati sufficienti per l'analisi della vitalità della popolazione (PVA). Il monitoraggio è stato condotto utilizzando un sistema a griglia con quadrati di 1 m², ulteriormente suddivisi in quadranti. I singoli ramet sono stati contati e classificati come ramet prostrati (vegetativi) o ascendenti (riproduttivi). Nel 2023, sono stati registrati 1159 ramet, comprendenti 949 individui vegetativi e 210 individui riproduttivi. L'area effettivamente occupata era di circa 18,75 m², con *L. inundata* documentata in 75 quadranti. La distribuzione era limitata a una stretta fascia parallela alla riva del lago Riondo, con densità più elevate osservate in aree con ridotta copertura da parte di altre specie e attorno alle cavità. La popolazione rappresenta una presenza significativa nella penisola italiana, dove la specie mostra una distribuzione relitta. Tuttavia, è stato confermato a livello regionale lo stato di conservazione in pericolo critico (CR) di *L. inundata* a causa del numero limitato di individui riproduttivi. La protezione e il monitoraggio forniti dai Carabinieri Forestali nella riserva sono stati efficaci nel conservare la specie impedendo la raccolta indiscriminata, il pascolo e il calpestio eccessivo.

Parole chiave - *Lycopodiella inunda*ta, Superficie coperta, densità, distribuzione, popolazione, Agoraie

Introduction

Lycopodiella inundata (L) Holub, the Marsh Clubmoss, is a isosporous perennial pteridophyte within Lycopodiaceae (Lycophyta; Holub, 1964). It is a small, bright green, creeping, moss-like plant that produces 2-3 cm tall cones with weakly differentiated sporophylls in autumn. The species has an amphi-Atlantic distribution and occurs in Japan, North America (Roskov *et al*., 2018), and central and northern Europe (Jermy, 1993; Prelli & Boudrie, 2021), but it is absent from the central parts of Russia (Rasmussen & Lawesson, 2002). In Italy, it is mainly found in humid depressions, sandy depressions, marshes, and peat bogs from 200 to 2000 m a.s.l. (Pignatti, 2017-2019); it is documented for only 15 sites between the Alps and Apennines (Troia & Greuter, 2015), and has undergone local extinction at several of these locations (Bona *et al*., 2005). *Lycopodiella inundata* is

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an endangered wetland species that can also thrive in secondary, anthropogenic habitats. A five-year study in Central Poland revealed significant changes in its occurrence and population dynamics (Kiedrzyński *et al*., 2016).
This plant species was first documented in the Agoraie Nature Reserve in 1976 (Pichi Sermolli, 1976). The earliest comprehensive documentation of the distribution of this population in proximity to Lake Riondo was provided in 1979 (Aita *et al*., 1979). The purpose of the present study was to characterize the population of Riondo Lake by examining specific parameters including the number of individuals (ramets), covered area, density and distribution. The aim is to acquire sufficient data over a minimum period of three years to conduct a Population Viability Analysis (PVA; Morris & Doak, 2002; Vaira, 2012).

Study area

The "Agoraie di Sopra e Moggetto Biogenetic Nature Reserve" was established in 1971 as a "full reserve" and then reclassified as "oriented" after 1986. It is situated in the municipality of Rezzoaglio, province of Genoa (Liguria, Northern Italy), between 1325 and 1360 m a.s.l (Fig. 1). The area included in the reserve represents the sole remaining evidence of the Quaternary glaciation in Liguria. The Reserve comprises two biotopes: Agoraie (approximately 15.50 ha) and Moggetto (approximately 1.40 ha). The former contains four perennial lakes: Lago degli Abeti, Lago Riondo, Agoraie di Mezzo, Agoraie di Fondo, and two seasonal water bodies: Pozza degli Abeti and Agoraie di Sopra also called Stagno Piccolo; the latter encompasses the seasonal Stagno Lagastro or Moggetto. The origins of the lakes can be traced to the Quaternary period, specifically to the last glaciation (Aita *et al*., 1979). The property is under state ownership, with management entrusted to the "Corpo Forestale dello Stato," which became "Carabinieri Forestali" after 2017, specifically the "Reparto Carabinieri Biodiversità di Lucca." The reserve is entirely enclosed with visitor access limited to 300 per year. *Lycopodiella inundata* was found exclusively in *Sphagnum* moss surrounding Lake Riondo; prior to 2008, it was considered the southernmost population in Italy (Camangi & Kugler, 2008). The plant is classified as critically endangered (CR) for Liguria (Conti *et al*., 1997), whereas it is classified as Data Deficient (DD) for Italy (Rossi *et al*., 2013) and globally as Least Concern (Lansdown, 2014). Peat bog ecosystems are classified as Vulnerable (VU) in the IUCN Red List of Italian ecosystems (Blasi *et al*., 2023). Active raised bogs are classified as Vulnerable (VU) in the European Red List of Habitats (European Red List of Habitats - enhanced by EEA, 2022).

Material and methods

The optimal period for analyzing the structure and size of plant populations is when the majority of reproductive individuals are in the fruiting stage (Iriondo *et al*., 2009; Morris & Doak, 2002). This period was identified in the first year (from 18 to 23 August), and subsequent surveys should be conducted on the

Figure 1. Study Area.

same days to incorporate environmental stochasticity (Iriondo *et al*., 2009). Initially, georeferenced benchmarks were established on the mainland (because the peat bog does not ensure the stability of the georeferenced positions over time) using global positioning system (GPS) equipment (GNSS South Mod. Galaxy G7) with sub-metric precision, to which the monitoring grid was anchored. These benchmarks were selected to encompass the entire population of *Lycopodiella inundata* and facilitate unambiguous annual population monitoring.

For the purposes of our study, given the small population size, we adopted a definition other than the Area of Occupancy *sensu* IUCN, whose grid of 4-km square cells would not have been useful. Therefore, we used a quarter of a square meter (quadrant) as the unit, and the surface area actually occupied by the species was called "Covered Area."

The monitoring grid comprised 1 × 1 m (1 m²) squares identifiable by x and y coordinates denoted by letters and numbers (e.g., A1, B2, etc.), further subdivided into four quadrants of 50 × 50 cm (2500 cm²) marked, from the top left in a clockwise direction, by Roman numerals (I, II, III, IV). The grid was incorporated as a new layer in a specific QGIS project (QGIS ver. 3.34.8, EPSG:6706 RDN2008) using benchmarks as reference points. Subsequently, the grid was materialized on the ground using graduated lines anchored to benchmarks.

The right angles (90°) necessary for constructing a perfect square on the ground were obtained using a Pythagorean triplet, with a vertex positioned at each benchmark.

To accurately enumerate ramets, a 1m² mobile square was utilized, constructed from Aluminium, and subdivided into four equal quadrants (Carah & Chávez, 2007; Vitt & Skogen, 2016) (Fig. 2). All species were identified in each quadrant, and each species was scored for ground cover. The degree of cover was measured as the vertical projection of all the aerial parts of plants of a given species as a percentage of the total plot area (Westhoff and Van der Maarel, 1978). The abundance of *L. inundata* was quantified using cover percentage, in accordance with the scale of Westhoff and Van der Maarel (1978), modified, and refined by subdividing the intervals at 5 percentage point increments, this subdivision allowed for a more precise rendering on QGIS. Every interval has a value (Cover Index), as presented in Table 1. Subsequently, these indices were transposed into the QGIS project. Furthermore, due to the impracticality of isolating a single genet of *Lycopodiella* without causing damage, we opted to enumerate ramets; therefore, the individual ramets of *L. inundata* were enumerated and categorized as either prostrate ramets (vegetative) or ascending ramets (reproductive, sporophyll-bearing; Figs. 3-4). For relatively small populations, this methodology facilitates highly accurate measurements of the number of individuals (Elzinga *et al*., 1999, 2019; Spireas, 2016) and Density (Efford & Dawson, 2012). Additionally, for each identified square meter, five high-resolution photographs were taken: one for the entire square and one for each of the four quadrants. A digital camera (Panasonic Lumix DMC-FZ1000) was used for this purpose. The photographs were subsequently transferred to a computer and labeled according to year, square and quadrant (e.g., 2023H7 for the photograph of the entire H7 square and 2023H7IV for the photograph of the H7 square, fourth quadrant).

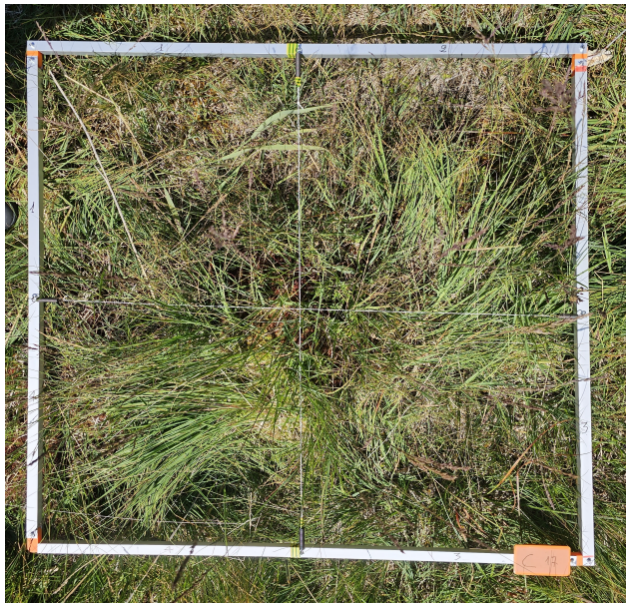

Figure 2. The square employed during monitoring.

Table 1. Scale for extimation of ground cover percentage in herbaceous and shrub vegetation (adapted and modified from Westhoff and Van der Maarel, 1978).

Value (Cover Index)	Percentage of cover
1	≤ 1%, 1 - 3 individuals
2	≤ 2%, 3 - 10 individuals
3	2 - 5%, ≥ 10 individuals
4	6 - 10%
5	11 - 15%
6	16 - 20%
7	21 - 25%
8	26 - 30%
9	≥ 30%

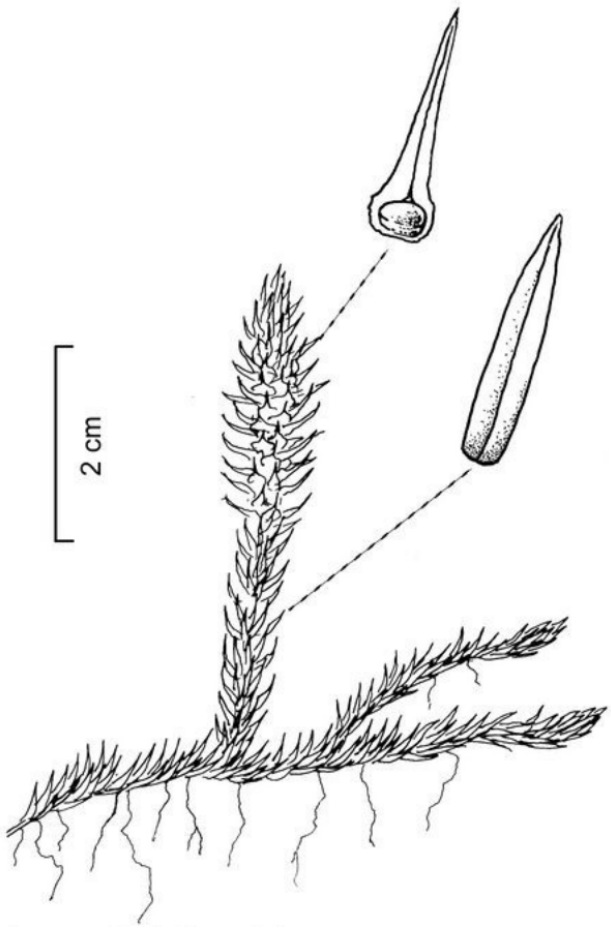

Figure 3. Illustration of prostrate ramets and ascending ramets of *L. inundata.*

Figure 4. Field photographs of prostrate and ascending ramets of *L. inundata.*

Results

Monitoring was conducted from August 19 to 21, 2023. It will be repeated annually during the same period.

Number of individuals and cover

The counting of individuals resulted in 1159 ramets, comprising 949 vegetative ramets and 210 reproductive ramets. Table 2 shows the number of individuals detected in 2023, divided into quadrants, ground cover percentage in each quadrant, Cover index, number of reproductive, vegetative ramets and total ramets.

Density

We observed that the density of *L. inundata* was much higher in 100% of the eight quadrants where small holes and cracks in the peat were present: G19 I, H14 I, H15 IV, K15 III, K15 IV, M12 I, P11 IV and T9 III. Conversely, the density was very low in the quadrants where the coverage of competing species was higher. A previous study conducted in the United Kingdom (Rasmussen & Lawesson, 2002) reached the same results.

Distribution

Fig. 5 illustrates a comparison between the findings of Aita *et al.* (1979) and those of this study. The distribution of *L. inundata* was observed to maintain approximately the same spatial pattern.

Covered Area

As depicted in Fig. 6, the covered area encompasses approximately 18.75 m^2, with *L. inundata* documented in 75 quadrants. This figure further illustrates the percentage of cover (density) and the distribution of the entire population calculated for each quadrant.
The orthophoto layer (custom July 2023) has been incorporated into Fig. 7, wherein it is possible to discern the nuances of the distribution and density.

Discussion

Although the population of *L. inundata* examined in this study is arguably one of the most significant in the entire Italian peninsula, the Covered Area is notably small, comprising only 1159 individuals.
The spatial distribution of the population observed by Aita *et al.* (1979), as shown in Fig. 4, is potentially subject to error, which is likely due to the manual

Table 2. Square ID; quadrant; % coverage in each quadrant (%LYC); Coverage Index (CI); Reproductive Ramets (RR); Vegetative Ramets (VR); Total Ramets (TR).

Square ID	Quadrant	% LYC	CI	RR	VR	TR
C17	I	<1	1	2	0	2
C17	II	5	3	12	15	27
C18	III	3	3	4	10	14
C18	IV	<1	1	2	1	3
D15	II	<1	1	0	1	1
D15	III	1	3	3	14	17
D16	III	<1	2	1	3	4
E17	I	<1	1	0	2	2
E17	II	4	3	0	12	12
E17	III	<1	3	2	9	11
E17	IV	10	4	5	24	29
E18	I	<1	1	0	3	3
E18	IV	<1	2	1	5	6
F17	I	<1	2	3	3	6
F18	II	<1	1	0	1	1
F18	III	1	2	3	7	10
F18	IV	<1	1	0	1	1
F19	I	1	1	0	3	3
F19	III	<1	2	0	4	4
F19	IV	2	3	2	18	20
G15	I	1	2	1	6	7
G15	IV	2	3	6	16	22
G17	II	1	2	1	5	6
G17	III	<1	1	0	2	2
G18	III	<1	1	0	1	1
G19	I	10	4	9	49	58
G19	II	<1	2	1	6	7
G19	IV	5	3	7	20	27
H13	I	2	3	4	14	18
H14	I	20	6	12	40	52
H14	II	<1	2	1	3	4
H14	IV	3	3	2	7	9
H15	III	6	4	6	15	21
H15	IV	30	8	13	72	85
I13	II	<1	2	2	2	4
I14	II	2	3	2	11	13
I14	III	5	4	4	16	20
I14	IV	<1	1	1	2	3
I17	III	<1	1	0	2	2
I17	IV	<1	1	0	3	3
J13	II	5	3	2	11	13
J15	I	5	3	6	15	21
J15	III	3	3	3	7	10
J15	IV	2	3	3	11	14
K12	II	<1	1	0	2	2
K15	I	10	4	3	25	28
K15	II	<1	2	0	4	4
K15	III	20	6	9	43	52
K15	IV	25	7	9	42	51
L13	I	<1	2	0	5	5
L13	II	<1	1	0	1	1
L13	III	<1	1	1	2	3
L13	IV	3	3	2	11	13
L14	III	<1	1	1	2	3
M12	I	20	6	11	33	44
M12	II	5	3	4	17	21
M13	III	20	6	6	45	51
M13	IV	3	3	1	12	13
P10	I	3	3	0	17	17
P11	I	1	3	3	13	16
P11	III	<1	2	2	8	10
P11	IV	20	6	3	46	49
Q10	I	<1	1	1	1	2
Q10	II	2	3	3	9	12
Q10	III	<1	1	0	2	2
R10	IV	<1	2	2	4	6
S9	I	<1	2	1	7	8
S9	IV	1	2	2	8	10
T9	I	3	3	0	20	20
T9	II	8	4	4	23	27
T9	III	25	7	10	55	65
T9	IV	<1	2	1	4	5
T10	IV	<1	2	2	7	9
U10	II	<1	1	1	2	3
U10	III	<1	2	2	7	9

nature of the survey conducted without the assistance of GPS technology. In this instance, the error appears to be systematic, as the distribution follows a trend consistent with our findings, but is displaced by several meters to the west.

The distribution detected by us appears to be confined to a narrow band that is on average just over 1 m wide with a maximum of 5 m approximately, and parallel to the shore of Lake Riondo. According to Rasmussen & Lawesson (2002), we observed a similar density distribution: in fact we also noted higher densities of individuals in areas with reduced cover by other species and around cavities, including a small peat clod that was created approximately twenty years ago for educational purposes (H15 IV, see Table 2). This observation opens the way to a possible method to increase the numerical consistency of a population of *L. inundata*. After the conclusion of the monitoring phase, with prior authorization from the "Reparto Carabinieri Biodiversità" di Lucca, we intend to experimentally verify its validity.

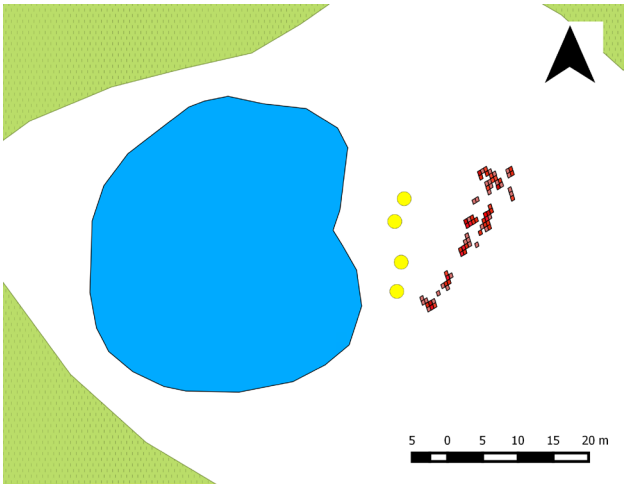

Figure 5. Yellow dots indicate the distribution of *L. inundata* observed in 1979, red squares indicate the distribution of *L. inundata* observed in 2023.

Figure 6. Covered area, density and distribution of *L. inundata*.

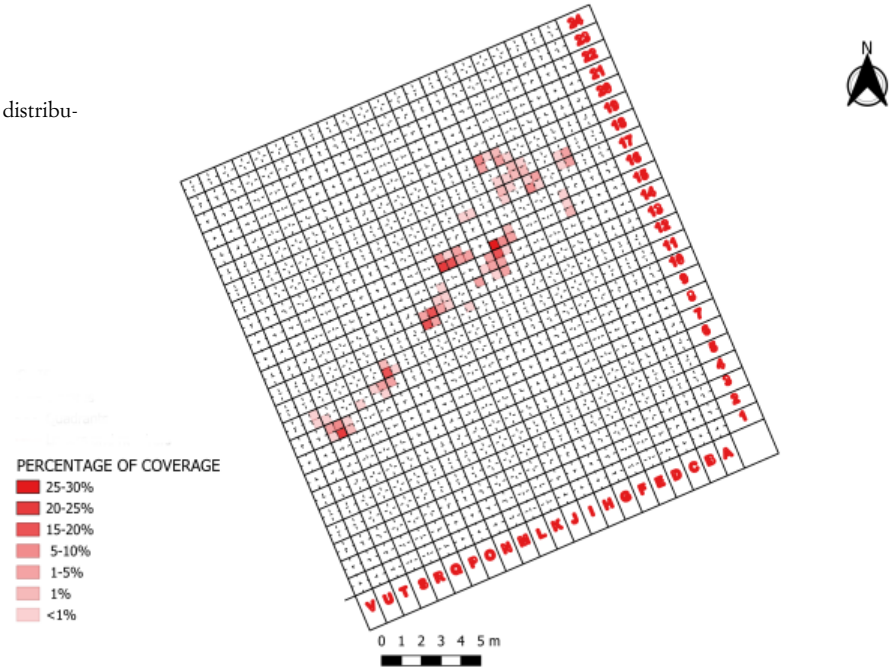

Figure 7. The distribution of *L. inundata* superimposed on the orthophoto.

Conclusions

A comprehensive investigation of a *Lycopodiella inundata* (L.) Holub population was conducted for the first time in Italy, as a starting step for a long-term monitoring program. Initial findings indicate that the IUCN conservation status of this species remains critically endangered (CR) at the regional level because of its small number of reproductive individuals and because the population is the only one found in Liguria (IUCN, 2012).
A notable advantage is that the reserve is enclosed, safeguarded, and monitored by the Carabinieri Forestali, who effectively supported the conservation of the species by preventing collection, grazing, excessive trampling, and anthropogenic drainage of water, which are factors that potentially contribute to the local extinction of certain populations (Rasmussen & Lawesson, 2002; Bona *et al*., 2005; Kiedrzyński *et al*., 2016).

Conflict of interest

The authors declare that they have no conflicts of interest.

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