BRYOPHYTES IN LIGURIAN 4090 HABITAT: ENDEMIC ORO-MEDITERRANEAN HEATHS WITH GORSE (*GENISTA DESOLEANA*)

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ABSTRACT
Despite Mediterranean vegetation is largely investigated, the knowledge about the Bryophytes living in this highly heterogeneous environment is still scarce. For some habitat, such as the endemic oro-Mediterranean heaths with gorse (Natura 2000 habitat 4090), no data on Bryophytes are available. We investigated the Bryophytes occurring in twelve *Genista desoleana* hedgehog-heaths in eastern Liguria (NW Italy). We proposed a new method for sampling mosses in arid habitats with poor bryophytic coverage. Moreover, we provided the first preliminary checklist of Bryophytes occurring in the Ligurian 4090 Habitat. As expected, Bryophytes showed very low diversity and coverage, mainly forming small colonies hidden under the shrubs. Several stress-tolerant and ruderal taxa were found, and *Weissia controversa* Hedw. var. *controversa* resulted the most frequent and abundant species.

KEYWORDS: Bryophytes, checklist, *Genista desoleana*, heaths, Liguria, Mediterranean, ophiolites, sampling, 4090.

INTRODUCTION

The Ligurian Mediterranean vegetation, often characterized by shrubs and heaths, is a very remarkable and peculiar object of study because of the many singular and atypical aspects of the region, regarding geology, geomorphology, climate and geographical position (Barberis et al., 1997). In particular, the *Genista desoleana* hedgehog-heaths are a typical Ligurian Mediterranean habitat, mainly occurring on the ophiolitic outcrops of the eastern part of the region, characterized by xerophile to mesoxerophile formations with a discontinuous, medium to low vegetation coverage (Mariotti 1994, 2008; Vagge, 1997). This vegetation belongs to the association *Euphorbio spinosae-Genistetum desoleanae* Nowak 1987 corr. Vagge 1997 and is the Ligurian expression of the Natura 2000 habitat 4090 “Endemic oro-Mediterranean heaths with gorse” (Mariotti, 2008), often characterized by the presence of the Italian endemics *Genista desoleana* Valsecchi and *Santolina ligustica* Arrigoni (Vagge, 1997).

This habitat is considered of high scientific interest because of its floristic and vegetational peculiarities, also due to the ophiolitic substratum (Mariotti, 2008). Consequently, many studies have been performed on these plant communities in Liguria, where the habitat reaches its northern distributional limit (Furrer & Hofmann 1969; Nowak, 1987; Mariotti, 1994; Vagge, 1997). Nevertheless, to the best of our knowledge no data about the bryophytic component of this habitat are available. This condition is shared with several Mediterranean habitats, since in Mediterranean region Bryophytes are much less studied than vascular plants (Geissler, 2001;

Murru et al., 2018). Additionally, in Ligurian region only a scarce number of studies on Bryophytes have been performed, and the level of knowledge about Bryophytes is quite below the national average, leading to an underestimation of the number of bryophytic taxa occurring in this region (Poponessi et al., 2014). Considering the vegetation structure and the environmental features of the habitat 4090, the bryophytic coverage in this habitat is expected to be low, as already observed by Mariotti (1994), probably explaining why botanists have often ignored them.

There is little agreement about the sampling methods for Bryophytes, and standard protocols are often lacking in literature (Berg et al., 2016), in spite of the general recommendation of taking into account all the recognizable microhabitats during the sampling of Bryophytes (Loeske, 1925; Raup, 1926; Newmaster et al., 2005). The absence of uniformity could be a contributing cause to the lack of attention paid to Bryophytes during vegetation studies within habitat of Community interest, where Bryophytes are often sampled with the same methods as vascular plants or which are not sampled at all (Berg et al., 2016), leading to an underestimation of rare and small species (Vanderpoorten et al., 2010).

All these considerations prompted us to propose a new bryophyte sampling method for the habitat 4090, which can be effective in sampling mosses in arid habitats with poor bryophytic coverage. Moreover, the aim of this study is also to provide the first checklist of the Bryophytes occurring in this habitat in Liguria.

STUDY AREA

We selected twelve sites where the target habitat occurs within the eastern Ligurian Apennines (Fig. 1). All the selected sites show a *Genista desoleana* dominated hedgehog-heat on ultramafic substrate, but they are variable in terms of other stational features, being representative

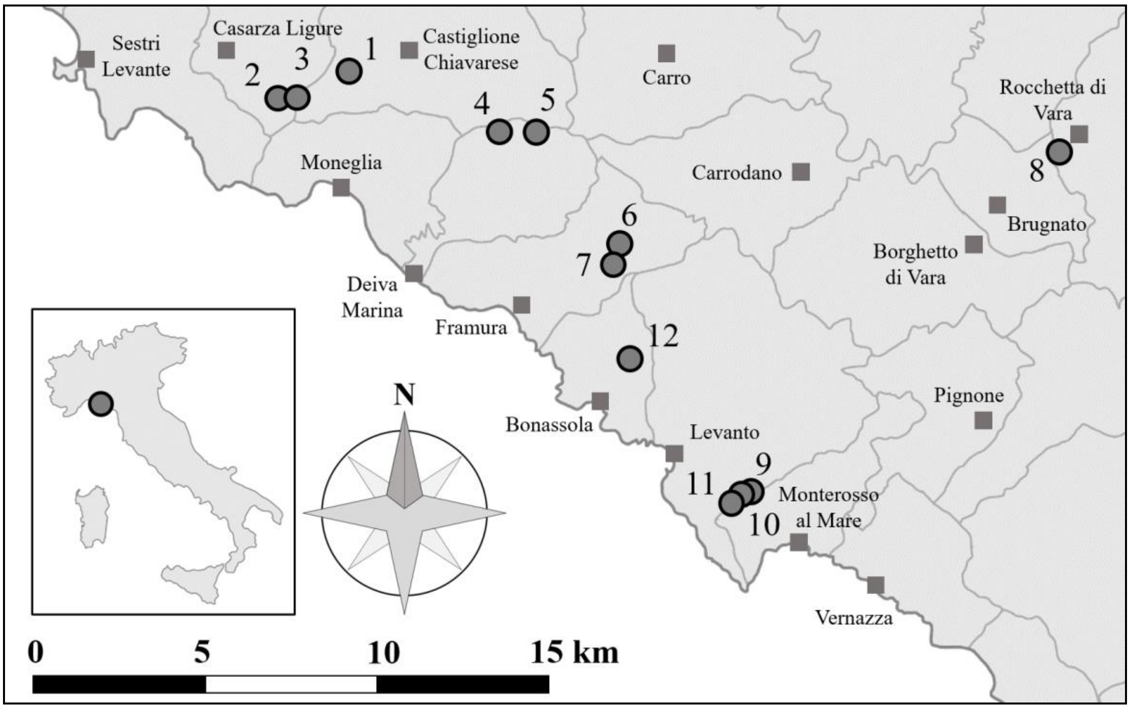

Figure 1: Map of the study sites.

Table 1: The study sites. In the first three sites we applied the sampling method proposed in this paper, while in the other sites we performed a simple sampling. Most sites are located in a Natura2000 Special Area of Conservation (S.A.C.). Coordinates are expressed in WGS84 EPSG:4326. Altitude is expressed in meters above sea level.

Code	Locality (Municipality)	S.A.C.	Latitude; Longitude	Altitude	Bibliographic sources
1	Masso (Castiglione Chiavarese)	IT1333307	44.270109; 9.492761	295	/
2	Monte Croce dei Tozzi, W side (Casarza Ligure)	IT1333307	44.263086; 9.467245	224	/
3	Monte Croce dei Tozzi, W side (Casarza Ligure)	IT1333307	44.263400; 9.474050	301	/
4	Monte Merelle (Deiva Marina)	IT1343412	44.252477; 9.546981	508	Mariotti 1994
5	Passo del Bracco (Deiva Marina)	IT1343412	44.252229; 9.559655	612	Mariotti 1994, Vagge 1997
6	Foce di Vaggi (Framura)	IT1343415	44.223780; 9.591281	586	Vagge 1997
7	Colle Guaitarola (Framura)	IT1343415	44.218220; 9.588199	605	Mariotti 1994, Vagge 1997
8	Rocchetta di Vara (Rocchetta di Vara)	/	44.247647; 9.750686	207	Mariotti 1994, Vagge 1997
9	Monte Nero, SE side (Monterosso Al Mare)	IT1344210	44.15894; 9.63714	407	/
10	Monte Nero, SE side (Levanto)	IT1344210	44.15851; 9.63568	436	/
11	Sella de Bagari (Levanto)	IT1344210	44.15581; 9.63102	355	Mariotti 1994
12	Rossola (Bonassola)	/	44.193572; 9.594979	372	/

of the geographical and ecological variability of the habitat in the study area (Tab. 1). Some of the selected sites were subjected to past floristic studies, focused on vascular plants (Mariotti, 1994; Vagge, 1997).

MATERIALS AND METHODS

The following protocol for sampling of Bryophytes has been applied to the sites 1, 2 and 3 (Tab. 1). In each site, we delimited a sampling square of area 25 m^2, according to the Italian monitoring protocol of 4090 habitat (Angelini et al., 2016). In this area, a floristic relevé was performed, listing all the species occurring within the sampled area. We provided a percentage coverage value for each vegetation layer (i.e., bryophytic, herbaceous, shrubby and arboreal) and vascular species. Coverage values were assigned as multiples of 5, with the exception of the 1% value; in the case of coverages less than 1%, we used the "+" symbol. To detect the Bryophytes species composition and the coverage values of each species we were partially inspired by the sampling method used for grassland's studies (e.g., Daget & Poissonet, 1969). First, we divided the square of 25 m^2 into five bands measuring 1x5 meters (Fig. 2). Then, using a tape measure, we plot a line in the center of each band, running in a median position along the bands parallel to their longer side. The median lines were analyzed continuously, sampling all the Bryophytes no more than 10 cm away from either side of the line, then the sampling area was made of five bands of 0,2x5 meters. Species occurrences were listed each times a bryophytic colony (e.g., turfs, cushions, mats, etc.) was observed along the bands, while specimen collections of the same species were made only if they occur in different bands and/or microhabitats, up to a maximum of three samples for each combination. To convert the number of occurrences into coverage values, we calculated the individual species contribution (C) according to the following formula: $C = (N_i/N_{tot})\times100$, where N_i is the number of the occurrences of the species and N_{tot} is the total number of occurrences of all the Bryophytes. Then, we calculated the coverage value corresponding to each species contribution compared to the total coverage of the bryophytic layer.

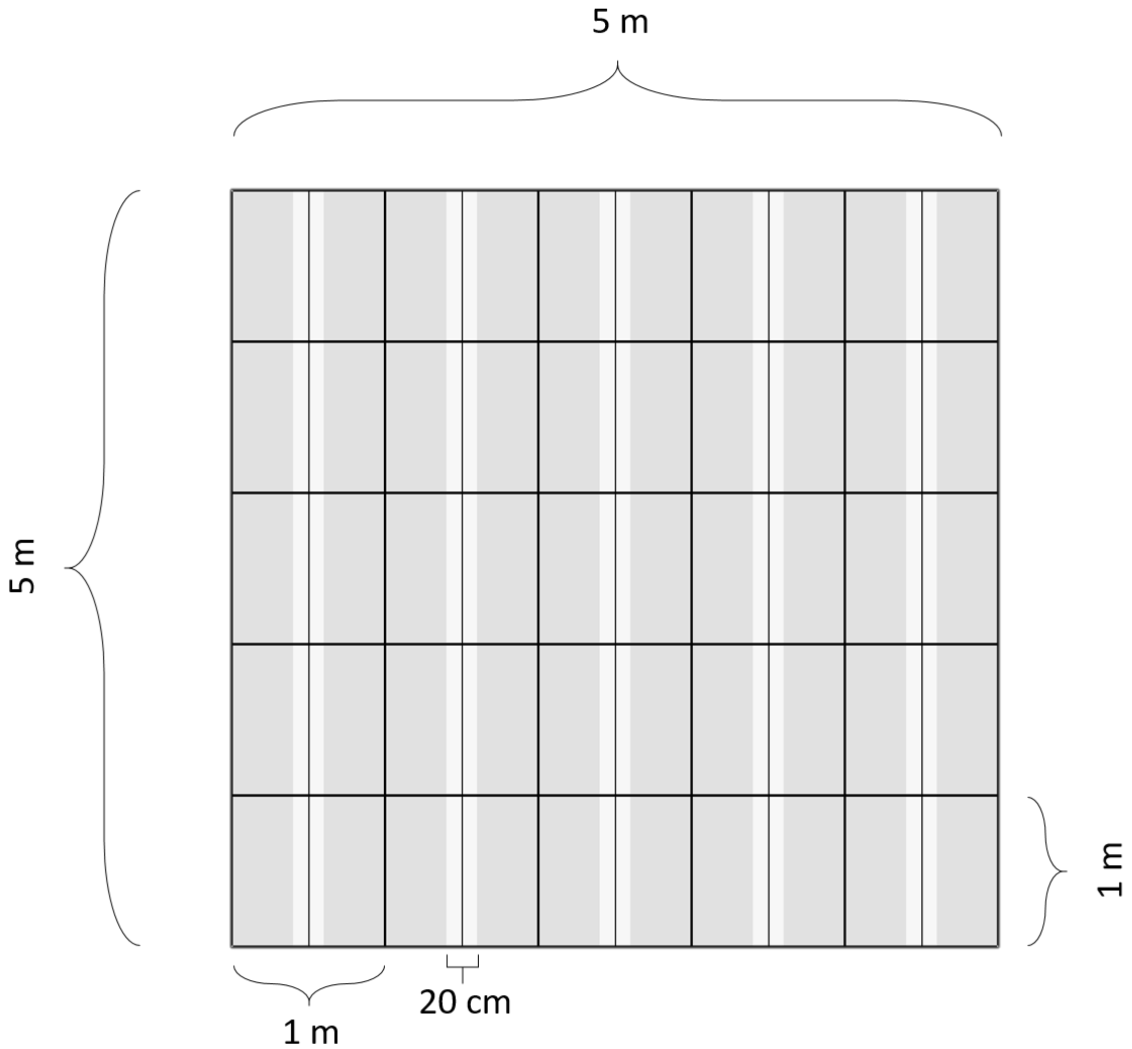

Figure 2: The sampling design of the method proposed in this paper. The white bands correspond to the sampled area for Bryophytes.

In the other study sites, we performed a simple Bryophytes sampling, randomly inspecting the sites and sampling the mosses we found. Together with data obtained from the three previous sites, these samplings allowed us to draw up a preliminary checklist of the

Bryophytes occurring in the 4090 habitat in eastern Liguria. For the checklist we followed the nomenclature by Aleffi et al. (2020). For each species, at least one herbarium specimen was deposited in GE.

RESULTS

The sampling method

The floristic relevés performed in the first three studied sites showed low values of overall floristic richness (i.e., always lower than 20 taxa); the vascular flora showed a higher number of species (i.e., ranging from 6 to 16) than the bryophytic flora (i.e., ranging from 2 to 4) in all sites (Supplemental material 1). The overall vegetation coverage was low in the three sites (i.e., ranging from 30 to 40%); despite the majority of vascular species were hemicryptophytes or therophytes, a few shrubby species (mainly chamaephytes and nano-phanerophytes) made up the majority of the overall vegetation coverage (Supplemental material 1). Conversely, the cover value of the bryophytic layer did not exceed 1% in any of the sites, and a single moss species (i.e., *Weissia controversa* Hedw. var. *controversa*) made up almost the total coverage of the layer in two out of three sites (Supplemental material 1). Despite the Bryophytes occurred in several micro-environments, the majority of samples were collected on soil sheltered by shrubs or rocks (Supplemental material 3). Predictably, the vascular component was mostly represented by thermophytic or barely cryophitic species occurring in dry and illuminated habitats (Supplemental material 1). By contrast, the bryophytic flora was largely formed by species having a wide tolerance range in light, temperature and humidity (Supplemental material 2). In both vascular and bryophytic components, stress-tolerant and ruderal life strategies were most represented.

The checklist

Overall, in the twelve sampled sites we found eleven species of Bryophytes (Tab 2; Supplemental material 2). The most recurring species was by far *Weissia controversa*, which occurs in the majority of sites. Most of the other Bryophytes were found only once. We mainly found species characterized by a wide tolerance in humidity, temperature and light, despite some species requiring dry, hot and illuminated habitats occur (e.g., *Weissia controversa* and *Grimmia laevigata* (Brid.) Brid.). The most common life form was the short turf, while among life strategies colonist-ruderal and perennial-stress tolerant species were more prevalent than competitive ones.

Table 2: The checklist of the Bryophytes of the Ligurian *Genista desoleana* hedgehog-heaths, with the occurrences per site.

Species	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**Frequency**
Weissia controversa Hedw. var. *controversa*		x	x	x	x	x	x	x					7
Grimmia trichophylla Grev.		x	x				x						3
Bryum sp.	x			x									2
Campylopus										x	x		2

Species	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**Frequency**
introflexus (Hedw.) Brid.													
Fissidens dubius P.Beauv. var. *dubius*			x										1
Hypnum cupressiforme Hedw. var. *cupressiforme*	x												1
Dicranum scoparium Hedw.	x												1
Cephaloziella divaricata (Sm.) Schiffn. var. *divaricata*	x												1
Grimmia laevigata (Brid.) Brid.									x				1
Ptychostomum capillare (Hedw.) Holyoak & N.Pedersen												x	1
Trichostomum brachydontium Bruch												x	1

Discussion and Conclusions

The sampling method

The new sampling method applied in the three study sites showed a good performance to obtain both a list of bryophytic species occurring in the vegetation plot and an assessment of their coverage values. In fact, most of the sampled species were small, difficult to observe and hard to distinguish on field (Supplemental material 3), as expected in a dry and hot habitat (Glime, 2017). In addition, the overall low bryophytic coverage value, already reported for the habitat 4090 in Liguria (Mariotti, 1994), makes difficult to assess quantitative differences among the species. For these reasons, our sampling method is probably better than the simple sampling approach, that, likely a random sampling, is effective in analyzing the frequency of the most common species, but it could lead to overlook rare and small species (Vanderpoorten et al., 2010), missing important variations within the sampled area (Slack, 1984; Newmaster et al., 2005). On one hand, our choice to follow the standard protocol for vascular plant sampling in the 4090 habitat (Angelini et al., 2016) assured a better integration of the bryophytic data to those on the vascular plants, since the use of the same standard area for sampling is considered a key factor for this purpose (Berg et al., 2016). On the other hand, we divided this area into smaller cells to assure a better sampling of Bryophytes, as suggested by many authors (Vanderpoorten et al., 2010; Berg et al., 2016). Moreover, we explicitly took into account the diversity of microhabitats, that is recognized as a main requirement to correctly investigate bryophytic communities (Loeske, 1925; Raup, 1926; Newmaster et al., 2005), since mosses are more strongly correlated to meso- and microhabitat conditions than vascular plants (Vanderpoorten et al., 2010; Glime, 2017).

A main limit of our sampling method was to assign a unitary coverage value for each observed moss colony, without considering their actual coverage area. Usually, the application of a systematic sampling at regular distances may overcome this issue, as showed for grass vegetation (e.g., Daget & Poissonet, 1969). However, the low moss coverage in the investigated habitat makes this solution inappropriate because of the risk of sampling areas mostly with no mosses, compromising the overall floristic list (Vanderpoorten et al., 2010). Thus, our simplification seems to be acceptable as the total coverage of the bryophitic layer in each sites was very low (i.e., no more than 1%), and there were no evident differences in size between the sampled bryophytic colonies. Certainly, in more moss-rich environments this choice should be reconsidered.

The checklist

Combining our sampling method with the simple sampling performed in the other sites, we obtained the first checklist of the mosses of the Ligurian 4090 habitat (Tab. 2; Supplemental material 2). Despite our data must be considered a preliminary result, the low number of sampled species (i.e.: 11 taxa) suggests a scarce bryophytic richness in the 4090 habitat. This low bryophytic richness might be linked to the low overall bryophytic coverage in the studied habitat, a feature observed by Mariotti (1994) and confirmed by our data (Supplemental material 1). In fact, a positive correlation between abundance and biodiversity among Bryophytes is well documented (Økland & Økland, 1996; Økland, 2000; Bergamini et al., 2001; Vanderpoorten & Goffinet, 2009; Vanderpoorten et al., 2010; Glime, 2017). Moreover, this habitat is mostly characterized by a discontinuous and low coverage of the shrub layer (Mariotti, 1994; 2008; Vagge, 1997), which reduces the possibility of Bryophytes growth. In such dry and exposed habitats, the bryophytic coverage is usually low and the colonies are often small (Wiklund & Rydin, 2004; Vanderpoorten & Goffinet, 2009; Glime, 2017). In addition, several types of disturbances, which remove shrub cover and periodically renew the soil exposure to climatic agents, can promote a further reduction of the bryophytic richness, as observed in other dry heathlands (Chytrý et al., 2001). This is a recurring feature of the habitat 4090 in Liguria, because it often occurs in disturbed areas (e.g., proximity of roads or slopes with substrate instability), as observed by Mariotti (1994; 2008) and confirmed by the finding of some ruderal species in both the vascular and the bryophytic layers (Supplemental material 1; Supplemental material 2). According to these expectations, the majority of Bryophytes were observed on soil covered by shrubs. This aspect can be explained by the need of mosses to defend against excessive drying (Vanderpoorten & Goffinet, 2009; Glime, 2017): the soil covered by vegetation provides a shelter from direct light (Virtanen et al., 2015) and from the overheating of the dark-coloured ophiolithic rocks (Vagge, 1997). This finding further agrees to the well-recognized fundamental role played by microenvironmental differences in temperature and humidity in the distribution of mosses (Vanderpoorten & Goffinet, 2009; Vanderpoorten et al., 2010; Glime, 2017). Conversely, the importance of the ophiolitic substrate in determining the observed poor bryophytic richness might have been low: while vascular plants growing over serpentine usually have a strong specialization (Brady et al., 2005), few specialist Bryophytes for this environment are globally known (Virtanen et al., 2015). However, we found some mosses that are known to live on this substrate as well, such as *Weissia controversa* (Virtanen et al., 2015), *Grimmia laevigata* (Puglisi et al., 2016) and *Hypnum cupressiforme* Hedw. var. *cupressiforme* (Bargagli et al., 2001).

As expected by the environmental features of the study sites, the vascular flora was mainly composed by thermophytic or barely cryophitic species living in illuminated and dry habitats (Supplemental material 1). Instead, the bryophytic component was mainly composed by species showing a wider tolerance range in humidity, temperature and light (Supplemental material 2). This result reflects a more generalist attitude of Bryophytes compared to vascular plants, which are usually much more specialized than mosses, especially in harsh habitats (Vanderpoorten & Goffinet, 2009). Nevertheless, according to the bryophytic life form classification proposed by Mägdefrau (1982), we found a strong predominance of mosses having a short turf life form (Hill et al., 2007; Glime, 2017), that is considered strongly related to dry (Kürschner, 1994), photophytic and thermophytic habitats (Birse, 1958). Regarding life strategies, colonist and perennial species (sensu During 1992) were the most abundant: the former have a potential lifespan of few years and numerous spores, thus showing a "ruderal" life strategy; the latter have a potential lifespan of many years, numerous spores and a low reproductive effort, suggesting a "stress-tolerant" strategy (Dierßen, 2001). Conversely, competitive taxa were less abundant.

Weissia controversa was by far the most common species, occurring in 7 out of 12 sites. This species colonizes soil or rocks in exposed, often disturbed environments (Cortini Pedrotti, 2001); it can grow in moderately to considerably dry habitats, in illuminated sites, and it is a considerably thermophytic species (Dierßen, 2001). This species is common in Italy, from the basal to the alpine zone (Cortini Pedrotti, 2001), and it is present in almost all the Italian administrative regions (Aleffi et al., 2020) and in many European countries (Ros et al., 2013). *Weissia controversa* was found in other Italian dry heaths, such as in Apulian garrigues, where occurs in particularly disturbed sites (Puglisi et al., 2019). This species was also found in all the 27 sites of a study on coastal Bryophytes of the Northern Mediterranean (Sabovljević & Sabovljević, 2007) and in some Sardinian coastal dunes (Murru et al., 2018), showing a strong connection with Mediterranean dry habitats. According to the Synopsis of the Italian Bryophyte Vegetation (Puglisi & Privitera, 2012), *Weissia controversa* var. *controversa* is a characteristic species of the Alliance *Grimaldion fragrantis* Šmarda et Hadàc 1944, which occurs in oligotrophic and xerophytic habitats, on dry and basic soil, mostly in disturbed sites (Puglisi & Privitera, 2012).

Despite the majority of taxa were terricolous, we found some saxicolous species, such as *Grimmia trychophylla* Grev. and *Grimmia laevigata*. Both species live on rocks from the basal to the mountain zone (Cortini Pedrotti, 2001), showing a wide tolerance range although the latter is more strongly associated to illuminated, hot and dry habitats (Dierßen, 2001). Both species are common in Italy, but *G. laevigata* was no longer recorded in Liguria according to Aleffi et al. (2020), so our find represents a confirm for this species in this region (an herbarium specimen was deposited in GE, with the code B460). These saxicolous taxa belong to the Alliance *Grimmion commutatae* v. Krusenstjerna 1945, which occurs in xerophytic to meso-xerophytic habitats, on hilly and montane belts (Puglisi & Privitera 2012). They seem to be more linked to the small outcrops that are frequently found in our study sites, than to the *Genista desoleana* heathlands.

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