Join our sampling campaign on *Hygrophorus* spp. associated with firs





Project FunDive

In FunDive we work towards putting fungal diversity on the map to enhance European conservation efforts. Fungi are essential for our ecosystems but have often been neglected in monitoring efforts and conservation practices, leaving them vulnerable to threats and habitat loss. We would like to engage you to change this.



For more information, please visit <u>https://fun-dive.eu/</u>

FunDive is a pan-European initiative funded by Biodiversa+ that brings together 33 partners in 22 countries to improve fungal monitoring across the continent. The goal of FunDive is to close the knowledge gap dealing with fungal distributions to improve fungal conservation using the help from you and other citizen scientists.

Why is fungal monitoring important?

Fungi are generally under-studied. Their global distribution patterns are poorly resolved. Also in Europe, despite centuries of fungal research, there is a lack of the distribution patterns of many fungal species. However, this knowledge is very important for effective conservation practices. For example, assessments of species for the IUCN Red List require an understanding of the distribution of said species.

What can you do?

FunDive is structured in different projects, each focusing on a specific target group of fungi. You can engage in each project by documenting and collecting fungal specimens. The process is simple:

- find a representative of a target species from project list: <u>https://fun-dive.eu/get-involved/current-projects/</u>
- make a photo and record your specimen in PlutofGO app <u>https://plutof.ut.ee/go</u> following our instructions <u>https://fun-dive.eu/get-involved/how-to-engage/</u>
- send it to your national point of contact <u>https://fun-dive.eu/get-involved/fundive-national-points-of-contact/</u>
- your specimen will be processed and identified based on molecular information
- you can follow your fungus on FunDive records: <u>https://fun-dive.eu/dataportal/</u>.

For more information on how to document your records, please visit <u>https://fun-dive.eu/get-involved/how-to-engage/</u>





Hygrophorus marzuolus (Fr.) Bres. and Hygrophorus associated with Abies spp. stands

is one of the targets for the 2025 FunDive projects which aim to investigate fungal biodiversity and distribution patterns in Europe

The genus *Hygrophorus* (Hygrophoraceae, Agaricales) comprises species commonly found in the temperate regions of the Northern Hemisphere (Huang et al., 2021; Tedersoo et al., 2009) and are distinguished by features such as a slightly sticky to slimy cap, adnate to decurrent gills, and a divergent hymenophoral trama (Arnolds, 1990; Candusso, 1997; Hesler and Smith, 1963; Lodge et al., 2014). Most of *Hygrophorus* form symbiotic relationships with either broad-leaved or coniferous trees, a few, such as *Hygrophorus olivaceoalbus* Krieglst. ex Gröger & Bresinsky and *Hygrophorus penarius* Fr., are parasitic (Agerer, 2012; Marino, 2008).

Some of these species form ectomycorrhizal symbiosis with *Abies* spp. The genus *Abies* is classified in 10 sections, of which two include the circum-Mediterranean fir species (e.g. *Abies alba* Mill., *Abies cephalonica* Loudon, *Abies pinsapo* Boiss) mainly restricted to Southern Europe (Eckenwalder, 2009; Farjon, 2010; The Gymnosperm Database, 2025). Generally, the circum-Mediterranean firs occur in mountain habitats at altitudes of above 400 m, up to 2400 m on deeper acid soils with high water reserves (Aussenac, 2002). In fact, they are located in humid or even very humid climates with an annual precipitation over 700-800 mm, concentrated principally during the winter period (Awad et al., 2014; Schuck HJ et al., 2008; Tinner et al., 2013). These firs commonly form pure stands in their optimal habitat, while at the borders they can be mixed with other tree species, such as beech (*Fagus* spp.), deciduous and evergreen oaks (*Quercus* spp.), pines (*Pinus* spp.) and junipers (*Juniperus* spp.) (Arista et al., 1997; Kaya and Raynal, 2001).

Hygrophorus marzuolus (Fr.) Bres. primarily forms Among Hygrophorus, ectomycorrhizal associations with coniferous trees of the Pinaceae family, most commonly with Abies (alba, x borisii-regis) and Picea, but also with Pinus (Pinus mugo Turra, P. mugo subsp. uncinate (Ramond ex DC.) Domin, Pinus nigra J.F.Arnold, Pinus sylvestris L.) and Pseudotsuga menziesii (Mirb.) Franco (in France). Less frequently, it is associated with deciduous trees such as Fagus sylvatica L., Castanea sativa Mill. and Quercus spp. (Ainsworth et al., 2023; Fraiture and Otto, 2015). The fungus is almost exclusively found in natural or semi-natural forests and only rarely in plantations. It has been documented on calcareous, acidic, and sandy soils (Fraiture and Otto, 2015). This species is particularly noteworthy due to its distinctive ecological traits, including a vernal fructification period shortly after the first snow melts and a semi-subterranean growth habit (Boccardo et al., 2013; Candusso, 1997; Fraiture and Otto, 2015). Additionally, it holds culinary significance in certain countries. It is known that *H. marzuolus* is distributed across temperate Europe, particularly around the Pyrenees, Alps, and Carpathians (Fraiture and Otto, 2015; Hygrophorus marzuolus (Fr.) Bres. in GBIF Secretariat, 2023). Few records come from bordering areas in Greece (Zervakis et al., 1998) and recently it was found also in the United Kindom (Ainsworth et al., 2023). Nevertheless, the European distribution of *H. marzuolus* and other *Hygrophorus* associated with European Abies spp. formations (either pure or mixed) has not been specifically investigated to date. These include species such as the above-mentioned H. marzuolus (Fig. 1a), and Hygrophorus abieticola Krieglst. ex Gröger & Bresinsky (synonymous with Hygrophorus pudorinus s. auct. plur., non Fr.) (Fig. 1b), Hygrophorus





camarophyllus (Alb. & Schwein.) Dumée, Grandjean & Maire (Fig. 1c), Hygrophorus capreolarius (Kalchbr.) Sacc (Fig. 1d) and others. In FunDive, our primary sampling focus is to investigate the geographic distribution of H. marzuolus in different habitats, as well as investigate the biodiversity and distribution of Hygrophorus species associated with Abies spp. stands across Europe.



Figure 1 Exemplares of the most representative species involved in this study. a) Hygrophorus marzuolus (Fr.) Bres. (photo of Simone Graziosi); b) Hygrophorus abieticola Krieglst. ex Gröger & Bresinsky (synonymous with Hygrophorus pudorinus s. auct. plur., non Fr.); c) Hygrophorus camarophyllus (Alb. & Schwein.) Dumée, Grandjean & Maire; d) Hygrophorus capreolarius (Kalchbr.) Sacc. The last three photos were taken by Alessandro Valdagni

By reporting your findings, you will add to the knowledge of this species group and your records will be important contributions to nature conservation.



Hygrophorus genus

Taxonomy

The *Hygrophorus* genus belongs to the order Agaricales and the family Hygrophoraceae (Candusso, 1997; Wang et al., 2023). It was first described by Fries in the early 19th century with the type species *Hygrophorus eburneus* (Bull) Fr. Later, the taxonomic delimitation of the genus *Hygrophorus* has been gradually improving to be more congruent with phylogeny and biology of its members. The taxonomic concept of *Hygrophorus* has varied among mycologists. Some, like Quélet (1888), Hesler & Smith (1963), and Largent (1985), supported a broad definition (*Hygrophorus s.l.*), including related groups like *Hygrocybe* within the genus. Others favored a narrower definition (*Hygrophorus s.s.*). For instance, Kummer (1871) elevated three "tribes" to independent genera: *Camarophyllus, Hygrocybe*, and *Limacium*, while Karsten (1879) used the name *Hygrophorus* (s.s.) has gained widespread acceptance (Bessette et al., 2012; Candusso, 1997; Horak, 1990; Wang et al., 2018).

Moreover, the *Hygrophorus* genus represents the hygrophoroid clade, identified as one of the six major lineages within the Agaricales order through a phylogenetic study utilizing six DNA loci (Matheny et al., 2006). From a phylogenetic perspective, *Hygrophorus* is the sole genus within the tribe Hygrophoreae of the family Hygrophoraceae. It is closely related to the tribe Chrysomphalineae, which includes the genera *Aeruginospora*, *Chrysomphalina*, and *Haasiella* (Lodge et al., 2014).

Diversity and morphological characters

Globally, approximately 130 species of *Hygrophorus* were described (Wang et al., 2023). Species of this genus are predominantly distributed across the Holarctic and temperate zones of the Northern Hemisphere. They include diverse ectomycorrhizal fungi that form symbiotic associations with trees, primarily from the families Betulaceae, Fagaceae, and Pinaceae, within various forest ecosystems (Boccardo et al., 2013; Candusso, 1997; Wang et al., 2023).

The genus is distinguished by its characteristic morphological features (Fig. 2). The pileus is typically smooth to viscid in texture and exhibits a wide range of colors. The gills are waxy in appearance, generally adnate or decurrent or slightly so, and the spore mass is consistently white. The hymenophore has slender basidia, and divergent hymenophoral trama (Boccardo et al., 2013; Candusso, 1997; Lodge et al., 2014).



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Figure 2 Morphological features of *Hygrophorus* genus. a) Young basidiomata of *Hygrophorus marzuolus* (Fr.) Bres., with numerous soil debris adhering to their surface, highlighting the characteristic viscous texture of the pileus; b) Close-up of the gills, showing their waxy appearance and slight decurrence





The characteristics of the most well-known *Hygrophorus* spp. associated to *Abies* spp. stands are presented below: **Hygrophorus marzuolus** (Fr.) Bres. (Boccardo et al., 2013; Candusso, 1997)



Figure 3 *Hygrophorus marzuolus* Krieglst. ex Gröger & Bresinsky badisiomata found in an *Abies alba* Mill. forest from Trentino Alto Adige region in northern Italy. Photo of Marco Floriani.

Pileus: the pileus of *H. marzuolus* (Fig. 1a, 3) measures 3–10 (up to 15) cm in diameter and is initially hemispherical with an inrolled margin, it quickly flattens and eventually becomes depressed at the center, often with an undulating, irregular edge. Its surface is marked by humps and irregularities, partly due to its tendency to grow partially embedded in the soil. The color is predominantly gray, occasionally fading or marked with lighter or whitish spots, and rarely showing ochre-like tinges. Over time, it may darken to lead-gray or nearly black. The cuticle is thin, only partially separable, and its texture varies with humidity, ranging from slightly viscous to dry but never translucent.

Hymenophore: it consists of adnate to subdecurrent gills that are sometimes slightly arched. They are widely spaced, thick, and interspersed with lamellulae. Initially white, they turn gray and eventually blackish starting at the base, with the edges remaining slightly lighter.

Stipe: it measures $5-8 \times 2-4$ cm and is solid, robust, and short, often appearing cylindrical, curved, or irregular in shape. The upper portion is white and powdery, while the rest is marked by fibrils and striations, becoming ashen gray towards the base.

Flesh: it is abundant, firm, and hygrophanous, with a slightly fibrous texture. It is white, with grayish tones beneath the cap cuticle and along the cap edges. Its odor is faint but can become unpleasant in mature specimens, while the flavor remains sweet and delicate, contributing to its unique characteristics in the wild.





Microscopy: the spore print is white and the spores measure $6.1-7.7 \times 4.5-5.7 \mu m$. They are ellipsoidal in shape, smooth, and feature a prominent apiculus. The spores often contain a large central guttule (oil droplet), with some displaying multiple smaller guttules throughout.





Hygrophorus abieticola Krieglst. ex Gröger & Bresinsky (synonymous with *Hygrophorus pudorinus* s. auct. plur., non Fr (Boccardo et al., 2013; Candusso, 1997)



Figure 4 *Hygrophorus abieticola* Krieglst. ex Gröger & Bresinsky (synonymous with *Hygrophorus pudorinus* s. auct. plur., non Fr.) badisiomata found in an *Abies alba* Mill. forest from Trentino Alto Adige region in northern Italy. Photo of Alessandro Valdagni.

Pileus: the pileus of *H. abieticola* (Fig. 1b, 4) varies from 6 to 12 cm in diameter. It starts as globose when young, then it changes to a convex form before finally flattening out, often with a broad, low umbo at the center. The margin of the cap remains involute even as it matures. The surface of the pileus is notably glutinous or slimy when wet. Over time, as the cap dries, the surface becomes less sticky and develops a dry texture. The cuticle is distinctively strawberry pink when the mushroom is young, giving the species its characteristic appearance. However, as the mushroom ages, this vivid coloration fades unevenly into whitish patches, sometimes showing slight yellowish discoloration, particularly in older specimens. The cuticle can be peeled away up to two-thirds of the way towards the center. **Hymenophore**: it is composed of gills that are adnate to shortly decurrent. The gills are relatively thick and not closely spaced, with lamellulae interspersed among them. Their color is whitish, though they may occasionally display purplish-red spots, adding another distinctive feature to the mushroom's appearance.

Stipe: it measures $5-10 \ge 1-2.5 \text{ cm}$, is cylindrical, regular in shape, and robust in structure. The upper portion is adorned with fine, flocculose (woolly or scaly) textures, contrasting with its overall smooth appearance. Its color is generally whitish, blending harmoniously with the rest of the mushroom.





Flesh: it is fibrous and white, with a tendency to yellow slightly at the base of the stem as the mushroom matures. Its scent is not particularly noteworthy, but its taste is described as slightly bitter.

Microscopy: *H. abieticola* exhibits smooth, ellipsoidal to ovoid spores containing small guttules (oil droplets), measuring approximately $7-9 \times 5-6 \mu m$. The basidia are tetrasporic.





Hygrophorus camarophyllus (Alb. & Schwein.) Dumée, Grandjean & Maire (Boccardo et al., 2013; Candusso, 1997)



Figure 5 *Hygrophorus camarophyllus* (Alb. & Schwein.) Dumée, Grandjean & Maire basidiomata found in an *Abies alba* Mill. forest from Trentino Alto Adige region in northern Italy. Photo of Alessandro Valdagni.

Pileus: the pileus of *H. camarophyllus* (Fig. 1c, 5) varies from 5 to 15 cm in diameter, initially campanulate (bell-shaped), it transitions to a convex form and eventually flattens out, often developing an undulating, lobed margin. At its center, it displays a broad, low, obtuse umbo. The cap margin starts involute, gradually opening with age. Its surface is greasy when moist, becoming dry and fibrous over time. The uniform coloration of the cap is striking, ranging from a deep brown-black to almost sooty in appearance.

Hymenophore: the gills are decurrent, arcuate, spaced apart, and have a waxy or greasy texture typical of the genus. Their coloration is a clean white, providing a stark contrast to the darker tones of the cap and stem.

Stipe: it measures $6-8 \ge 1-2$ cm, ranges from cylindrical to club-shaped and is predominantly whitish in color. It is adorned with fine fibrils that match the darker hues of the cap. These fibrils are arranged densely enough to give the stem a sooty appearance, enhancing the fungus overall dark aesthetic.

Flesh: it is firm and whitish, maintaining a solid texture throughout. It has a mild flavor and is almost entirely odorless.

Microscopy: the spore print is white and the spores are smooth and range in shape from ellipsoidal to ovoidal. They measure approximately 7–8.5 μ m x 4.5–5.5 μ m.





Hygrophorus capreolarius (Kalchbr.) Sacc., Bres. (Boccardo et al., 2013; Candusso, 1997)

Figure 6 Hygrophorus capreolarius (Kalchbr.) Sacc found in an Abies alba Mill. forest from Trentino Alto Adige region in northern Italy. Photo of Alessandro Valdagni.

Pileus: the pileus of *H. capreolarius* (Fig. 1c, 6) varies from 3 to 8 cm in diameter and ranges from hemispherical-campanulate to flattened, with a broad, obtuse umbo. The margin is long involute, the surface smooth, finely fibrillose, slightly viscous, and colored wine-brown to purplish with darker spots.

Hymenophore: the gills are adnate to subdecurrent, spaced, and the same color as the cap. **Stipe**: it measures approximately $3-7 \times 1-1.5$ cm, cylindrical, tapering at the base, dry, solid at first but later hollow, with longitudinal brownish streaks on a more or less light purplish background, darkening with age and upon handling.

Flesh: it is pinkish cream to grayish, with a mushroom-like odor and a sweet taste. **Microscopy**: The spore print is white and the spores are characterized by dimensions of $6-7 \times 4-5 \mu m$.



Purpose of mission and how to contribute

The primary objective of this study is to explore the geographic range of *H. marzuolus* across Europe in diverse environments, with the goal of enhancing our knowledge of its ecological needs and distribution trends. This includes examining its associations with specific hosts, soil preferences, and its responsiveness to natural disturbances and forestry management practices. Furthermore, this work addresses the need to preserve H. marzuolus as a species and its habitats, since it is nationally red-listed in several European countries where it occurs. It is classified as Endangered (EN) in Austria, the Czech Republic, and Germany; Vulnerable (VU) in Hungary, Macedonia, Serbia, and Slovakia; and Near Threatened (NT) in Switzerland (Dämmrich et al., 2016; Dämon and Krisai-Greilhuber, 2017; Fraiture and Otto, 2015; Karadelev et al., 2024; Kasom and Milickovic, 2006). Additionally, since it has traditionally harvested from local population, it is legally protected in a few countries, including Croatia, Germany, Serbia, and Slovakia, and is locally protected or subject to harvesting restrictions in certain regions of Italy (Fraiture and Otto, 2015; Onofri, 2005). In fact, the species is confined to mature forests and has experienced a population decline over the past 50 years, likely due to the loss of its suitable habitat in old-growth forests and possibly as a result of nitrogen deposition (The Global Fungal Red List Initiative, 2025). In fact, the main host of *H. marzuolus*, silver fir (*A. alba*), show a strong dieback in the 1980s in its range of occurrence in Europe (Tinner et al., 2013). Particularly in the Italian Apennines, silver fir could be of interest as it is an important species in five Natura 2000 habitats (Bottalico et al., 2014). Specifically, the Hygrophorus community of silver fir artificial plantations, whether inside or outside its natural range, such as those in Sardinia and Tuscany (Bottacci et al., 2012; Gabbrielli, 2003; Senni, 1955; Vazzano et al., 2011), needs to be studied.

Additionally, the study focuses on the biodiversity of the *Hygrophorus* genus within *Abies* spp. stands. Considering the rarity of these species, their restricted habitats, and the scarcity of specific data on their distribution (The Global Fungal Red List Initiative, 2025), their protection could be an important mission for European biodiversity conservation initiatives. Since sampling will also take place during spring, an uncommon season for fungal fructification, and in specific habitats, we aim to map the diversity of potentially undescribed species and report the presence of these species in areas where they have not yet been reported (especially *H. marzuolus*).

Regarding *H. marzuolus*, it can be sampled in various habitats, not limited to *Abies*, as explore its host preferences is crucial. For other *Hygrophorus* species, the presence of *Abies* spp. is a mandatory criterion for site selection, although the co-occurrence of other tree species is acceptable up to 50% in an area (20 m radius) around the collection point. Since this study focuses primarily on the biodiversity of *Hygrophorus* species in *Abies* spp. stands, rather than their fructification abundance, it is preferable to explore the maximum number of different wooded regions to identify as many *Hygrophorus* species as possible.

The best choice is to visit habitats and *Abies* spp. stands that cover a wide spatial distribution, ranging from north to south and east to west of your country. To ensure comprehensive data collection, it is essential to document the characteristics of each sampling site using the PlutoF GO app. In the "Habitat description" section (Fig. 7), users should note:

> The most likely host tree species associated with the specimen;



- The percentage presence of Abies spp. and other tree species (with a minimum diameter at breast height of 10 cm) within a circular area (radius 20m) surrounding the specimen collection point;
- Successional stage of the tree formation (e.g., old, mature, young);
- > Natural disturbances (e.g., wind, wildfire, landslide).

Data should be formatted as in the following example:

WP3 Hygrophorus, host tree species: *Abies alba*, Abies alba: 80%, Fagus sylvatica: 20%, successional stage: mature, natural disturbances: landslide

The putative host tree species should be chosen based on the closest tree to the collected basidiomata and the co-occurrence of other tree species in the surrounding area. Selecting specimens from a pure area is preferable, to reduce errors in the host-tree hypothesis. Furthermore, it is necessary to complete the "Substrate Taxon" and "Substrate Type" fields to provide a detailed context for the collected specimens. Furthermore, adding pictures of each sampling area is required. Pictures should be uploaded to the specimen page in the PlutoF GO app.

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Figure 7 To register detailed information about the habitat of a specimen follow these instructions on the PlutoF GO app

The sampling will focus on two main fructification periods: spring (from the end of February to June, especially for *H. marzuolus*) and autumn, depending on the climatic conditions of each country. We encourage all professional and amateur mycologists (Citizen Scientists) to collect at least one sample of *Hygrophorus* during the spring.



Specimens will be registered using the PlutoF GO app and uploaded to the main server as soon as possible after the sampling expeditions to ensure the accuracy of collection timing. Each collector should complete an Excel table recording all the specimen IDs used for this project and submit it to us at the end of spring and autumn. The basidioma samples will be dried and preserved in labeled paper bags following the specimen collection guidelines provided for PlutoF GO app samplings (<u>https://fun-dive.eu/get-involved/how-to-engage/</u>).

For each specimen, comprehensive documentation through notes and photographs is well received, suggesting the following:

- Young and mature sporocarps in a moist condition, arranged in a well-organized manner suitable for mycological scientific publication;
- The color of both young and mature gills;
- Distinctive characteristics of the species (e.g. smell of fresh specimens);
- Pictures and measurements of spores.

All pictures should be uploaded to the specimen page in the PlutoF GO app, and notes regarding the aforementioned details should be entered in the "Remarks" field of the app.



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