


# Update on the current occurrence of two rare fern species in Sicily (Italy): *Dryopteris affinis* and *D. robusta* in the Peloritani Mountains

**Gregor Kozlowski**


Department of Biology and Botanical Garden, University of Fribourg, Chemin du Musée 10, CH-1700 Fribourg, Switzerland  
Natural History Museum Fribourg, Chemin du Musée 6, CH-1700 Fribourg, Switzerland ✉ 

Corresponding author

**Jens Freigang**

Tal 11, 88386 Bergatreute, Germany ✉

**Salvatore Pasta**

Institute of Biosciences and BioResources (IBBR), National Research Council (CNR), Via Ugo la Malfa 153, I-90146 Palermo, Italy ✉ 

**Riccardo Guarino**

Department STeBiCeF, University of Palermo, Via Archirafi 38, I-90123 Palermo, Italy ✉ 

**Francesco Anania**

Via Garrisi 184, I-98057 Milazzo, Italy ✉

**Luca Champoud**

Department of Biology and Botanical Garden, University of Fribourg, Chemin du Musée 10, CH-1700 Fribourg, Switzerland ✉ 

**Giuseppe Garfi**

Institute of Biosciences and BioResources (IBBR), National Research Council (CNR), Via Ugo la Malfa 153, I-90146 Palermo, Italy ✉ 

**Alessandro Silvestre Gristina**

Department of Earth and Marine Sciences (DISTEM), University of Palermo, Via Archirafi 22, I-90123 Palermo Italy ✉ 


**Corrado Marcenò**

Department of Chemistry, Biology and Biotechnology, Via dell'Elce di Sotto 8, I-06123 Perugia, Italy ✉ 

**Rosa-Maria Picone**

Department ChiBioFarAm, University of Messina, V.le Stagno d'Alcontres 31, I-98166 Messina, Italy ✉ 

**Alessandro Crisafulli**

Department ChiBioFarAm, University of Messina, V.le Stagno d'Alcontres 31, I-98166 Messina, Italy ✉ 

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**Abstract.** The *Dryopteris affinis* species complex has a predominantly European distribution. The taxonomy and distribution of the Mediterranean taxa belonging to this complex are poorly studied and not fully understood. This paper provides new information on the occurrence and distribution of two taxa belonging to the *D. affinis* complex in Sicily, following the 2021 discovery of a new population in the Peloritani Mts. in the Cataolo Valley. The new population is composed of approximately 15 individuals. Plant material (7 individuals) was collected in May 2024 along an altitudinal gradient ranging from 1070 to 1120 m asl. A detailed morphological investigation, genome size analysis and ploidy level estimation were conducted for all of the sampled plants. Two individuals were identified as belonging to the diploid *D. affinis* subsp. *affinis* var. *affinis*, whereas the remaining five individuals were identified as triploid *D. robusta*. The confirmation of the presence of *D. affinis* in Sicily and the first finding of *D. robusta* on the island proves the key role played by the Peloritani Mts. as a refuge for species with a mid-European and boreal range.

**Keywords:** Floristic investigation, genome size, plant morphology, ploidy level.

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## Introduction

### The *Dryopteris affinis* complex

The *D. affinis* species complex has a predominantly European distribution, radiating to North Africa

(Atlas Mountains) and Western Asia (Pontic Mountains, Transcaucasia and Alborz Mountains) (Fraser-Jenkins, 1984). The taxonomic revision made by Fraser-Jenkins (2007) is a key reference for scientists studying this group. The species of

the *D. affinis* complex are characterized by obligate apomictic reproduction (agamospermy) and include diploid and triploid (rarely tetraploid) taxa. Fraser-Jenkins (2007) classified them primarily into species, subspecies and varieties. Due to numerous new combinations and a multitude of synonyms, the taxonomy and nomenclature of the *D. affinis* complex is somewhat intricate and misleading. In other apomictic plant groups, the respective taxa are described at species level, such as in the genera *Rubus*, *Hieracium* and *Taraxacum*. This standard is followed by Sell & Murrell (2018), who define all taxa of the *D. affinis* complex occurring in Great Britain and Ireland at the species level; however, taxa restricted to the European mainland were not considered. Thus, in this study, our main references are the works of Jäger (2011) and Sell & Murrell (2018). However, it is important to note that not all of the taxa listed in this publication are currently accepted in official checklists or on online taxonomic platforms such as *Plants of the World* (powo.science.kew.org). This is especially the case of *D. robusta*, one of the focus taxa of this study, which has often been synonymized with *D. borrieri*.

### The *Dryopteris affinis* complex in the Italian flora

In Italy, representatives of this complex may be found in various regions, mostly thriving in moist, shaded environments, such as forest understories, riverbanks, and mountainous areas (Marchetti, 2017; Bartolucci et al., 2024). However, their distribution pattern is not uniform. The species complex is far more common in the northern regions and becomes progressively rarer in the south, where occurrence is limited to favorable habitats and microhabitats, such as ravines and wooded gorges. Traditionally, *D. affinis* taxa are divided into two groups: diploids and triploids.

In the Italian literature, the diploid taxa are often not differentiated into subspecies and varieties and are described as *D. affinis* (Lowe) Fraser-Jenk. s. str. Marchetti (2004) reported occurrences of the diploid taxa, especially along the Tyrrhenian regions of Italy. Other regions where *D. affinis* s. str. was recorded include Lombardy, Piedmont, Liguria, Tuscany, Campania, Calabria, Sicily and Sardinia, plus an unverified record from Latium (Marchetti, 2017). Recent investigations by one of the co-authors (Freigang & Zenner, pers. obs.) as well as Marchetti (2017) showed that at least three diploid taxa occur in Italy: (1) *Dryopteris affinis* subsp. *affinis* var. *affinis* (Fomin) Fraser-Jenk., observed in Liguria, Lombardy and Tuscany, (2) *D. affinis* subsp. *affinis* var. *disjuncta* (Fomin) Fraser-Jenk., observed in Piedmont, Liguria and Lombardy, and (3) *D. affinis* subsp. *punctata* Fraser-Jenk., detected in Lombardy. Additional occurrences of these infraspecific taxa should be expected in other regions of Italy where *D. affinis* s. str. has been recorded.

The following four taxa of the triploid *D. affinis* complex have been observed or are expected to occur in Italy: (1) *D. borrieri* (Newman) Tavel., (2) *D. robusta* (Oberh. et Tavel ex Fraser-Jenk.) P.D. Sell., (3) *D. cambrensis* (Fraser-Jenk.) Beitel et W. Buck subsp. *insubrica* (Fraser-Jenk.) Fraser-Jenk., and (4)

*D. lacunosa* S. Jess., Zenner, Ch. Stark et Bujnoch. The first two taxa are often not differentiated and are merged in the literature under the name *D. borrieri*. Bartolucci et al. (2024) recorded the occurrence of *D. borrieri* throughout much of the Italian regions, apart from Sardinia and Molise and with unverified occurrences in Aosta Valley, Umbria and Sicily. Recent investigations by one of the co-authors (Freigang & Zenner, pers. obs.) demonstrated that there are occurrences of *D. robusta* in Liguria, Lombardy, Tuscany and Piedmont. In contrast, *D. borrieri* s. str. has not yet been detected in Italy. The third triploid taxon, *D. cambrensis* subsp. *insubrica*, was observed in Italy in Marche (Marchetti, 2009), Friuli-Venezia Giulia, Veneto, Trentino-Alto-Adige, Lombardy, Aosta Valley, Emilia-Romagna, Liguria, Tuscany, and Sardinia (Bartolucci et al., 2024) and was further confirmed in Liguria, Lombardy, Tuscany and Piedmont (Freigang & Zenner, pers. obs.). Finally, the fourth taxon, *D. lacunosa*, was observed in Liguria, Lombardy and Tuscany (Jessen et al., 2011; Marchetti, 2017).

A detailed identification key for all known *D. affinis* taxa observed in Italy may be found in Appendix 2.

### The *Dryopteris affinis* complex in the Sicilian flora

The taxonomy and distribution of the Sicilian fern taxa belonging to the *D. affinis* complex are poorly studied and not fully understood. Most of the specimens collected on the island date back to the 19<sup>th</sup> and early 20<sup>th</sup> centuries, at a time when the available floras did not differentiate the *D. affinis* taxa from *D. filix-mas* (L.) Shott, as demonstrated by the synonyms *Polystichum filix-mas* (L.) Roth and *Aspidium filix-mas* (L.) Sw. Gussone (1845) recorded the species in the woodlands of Castelbuono on the Madonie Massif and in Caronia and Cannata on the Nebrodi Mts. (Figure 1). Twenty years later, Todaro (1866) listed several valid arguments that strongly doubted the identity of the Sicilian ferns. He pointed out that the arrangement, color and size of the sporangia were completely different from those of the 'typical' *Polystichum filix-mas* and that the shape of the pinnae was much narrower and more elongated. Similarly, Nicotra (1893) remarked that in the Peloritani populations of *Aspidium filix-mas*, the 'typical' form was missing, thus the highly variable ferns were assigned to f. *incisa* T. Moore [= var. *incisum* (T. Moore) Milde] and f. *deorsolobata* T. Moore [= var. *deorsolobatum* (T. Moore) Rey-Pailh.]. Additionally, Lojacono-Pojero (1909) remarked that the Sicilian plants referred to as *Polystichum filix-mas* are quite different from the Mid-European ones.

Between the end of the 19<sup>th</sup> century and the beginning of the 20<sup>th</sup>, the main national (Fiori, 1923) and regional (Lojacono-Pojero, 1909) floras exclusively reported *Polystichum filix-mas* and its variety *paleaceum*. *Dryopteris paleacea* (Sw.) C. Chr. was found to be an illegitimate synonym of *D. wallichiana* (Spreng.) Hyl. (POWO, 2024). Since this species, absent in Europe and Italy, shows greater morphological affinity with the group of *D. affinis* (parallel pinnules not diverging from each other) than with *D. filix-mas*, the specimens from the territory of Santa Lucia del Mela (Zodda, 1900, 1908; Figure 1) in the Peloritani Mts. should belong to the *D.*

*affinis* complex. Consultation of the Sicilian herbaria (the herbarium codes cited in Thiers, 2024) revealed that all specimens kept in PAL and previously attributed to *D. filix-mas* have been revised by D. Marchetti and classified as *D. affinis*. These specimens come from several localities in the territory of Castelbuono (Figure 1), such as Contrada Rosselli/Russeddi (Minà, VI.1846, PAL10105; Todaro, s.d., PAL10103), Todaro (1866), Canalicchio (H. Ross, VI.1888, PAL 10102; sample and stand referred to *D. borrieri* by Raimondo & Mazzola, 1984) and San Guglielmo (Raimondo *et al.*, 2004).

More recently, Rizzo (2021) acknowledged three taxa: (1) *D. affinis* subsp. *affinis* (without variety indication), (2) *D. borrieri* (also without further differentiation), and (3) *D. cambrensis* subsp. *insubrica*. According to Rizzo (2021) and the available literature (e.g., Fiori, 1923, 1943; Raimondo *et al.*, 1994, 2004; Marchetti, 2004; Schicchi, 2004; Giardina *et al.*, 2007), only the subsp. *affinis* occurs in Sicily with certainty (in the northern districts of Palermo and Messina), while the presence of the two other taxa (*D. borrieri* and *D. cambrensis* subsp. *insubrica*) is uncertain and requires further confirmation.

Following the recent discovery of a new population in the Peloritani Mts., this paper provides additional information on the occurrence and distribution of two taxa belonging to the *D. affinis* complex from Sicily. The plants are identified to the species, subspecies or variety level based on morphological investigations (Appendix 1) and genome size analysis.

## Material and Methods

### Geographical, geological and climatological setting

The Peloritani Mountains form the north-eastern tip of Sicily and are part of an independent geological unit known as the Calabrian-Peloritan Arc, which has a long and complex history, dating back to the Paleozoic era (Cirrincione *et al.*, 2011). The mountain range currently presents an overlaid structure (alpine type) and shows an increasing degree of metamorphism from north to south, in which various stratigraphic units can be recognized; it primarily consists of ancient crystalline basement rocks (mainly schists, gneisses, and granites) that are partially covered by sedimentary deposits from the Mesozoic and Cenozoic eras, which were pushed upwards due to tectonic shifts (Angi *et al.*, 2010; Appel *et al.*, 2011; Fiannacca *et al.*, 2013). The complex geological and tectonic history of this range is reflected by its rugged morphology; in fact, despite its relatively low elevations, the Peloritani range includes mountains, hills and deep valleys and culminates in Montagna Grande (1374 m asl).

Based on the bioclimatic classification proposed by Rivas-Martínez *et al.* (2004), the Peloritani Mountains are subject to a Mediterranean pluviaseasonal oceanic bioclimate, with thermotypes ranging from the low thermo-mediterranean to the supra-mediterranean and ombrotypes ranging from the semiarid to the lower humid (Bazan *et al.*, 2015).

The physiognomic diversity of this territory explains the remarkable variety of habitats and extraordinarily rich flora and vegetation (Guarino,

1998a; Sciandrello *et al.*, 2015). However, in the last century, anthropogenic disturbance has caused continuous and unrestrained fragmentation of natural habitats (Guarino, 1998b).

### Study site and short history of the discovery

In June 2021, one of the authors (FA) discovered a small stand of *Dryopteris* in a forest habitat. Due to their ecology and morphological characteristics, the newly discovered individuals were assigned to the *D. affinis* species complex. The ferns grow along the Vallone Cataolo, a tributary of the upper reaches of the catchment area of the Torrente Mela, for a stretch of approximately 200 meters at altitudes between 1050 and 1150 m asl. A constant water supply throughout the year and considerable shading give the local microclimate an exceptionally cool and humid character. The *Dryopteris* inhabit a mesohygrophilous gorge forest community referred to *Tilio-Ostryon* Brullo, Scelsi & Spampinato 2001 and *Aceri obtusati-Ostryetum carpinifoliae* Brullo & Marcenò 1985. The undergrowth of this community is characterized by the presence and abundance of large ferns, such as *Athyrium filix-femina* (L.) Roth and *Polystichum setiferum* (Forssk.) T. Moore ex Woyne. Other understory species include *Drymochloa drymeja* (Mert. et W.D.J. Koch) Holub, *Geranium versicolor* L., *Cyclamen repandum* Sm. and *Daphne laureola* L. These forest nuclei, referred to as priority habitat 9180\* (*Tilio-Acerion* forests of slopes, screes and ravines) by the EU Directive 92/43, have been observed at several sites along the ridge of the Peloritani Mts, which have a meso-mediterranean thermotype and a humid-subhumid ombrotype (Picone *et al.*, 2009). The region also features dripstone walls covered by mosses and herbs, such as *Rhizomnium punctatum* (Hedw.) T.J. Kop. and *Saxifraga rotundifolia* L., respectively. Inside the gorge, the plants of *Dryopteris* grow on brown forest soils rich in humus and constantly humid, often near springs characterized by shade-tolerant hygrophilous vegetation dominated by *Rhynchochoris elephas* (L.) Griseb. and *Carex remota* L.

### Plant collection

The newly discovered population of *D. affinis* s.l. consists of approximately 15 individuals. Plant material (7 individuals) was collected on the 19<sup>th</sup> of May 2024 along the Cataolo stream and the altitudinal gradient ranging from 1070 to 1120 m asl. For each plant, only one well-developed leaf (frond) was collected and dried as an herbarium specimen for further morphological investigation. Thus, the collection method was by no means destructive. All the plants collected were large and well-developed individuals with 5-15 leaves. Removal of one leaf hardly reduces spore production and is compensated the next year without damage. The whole plant was photographed to document the morphology and structure of the living plants. Furthermore, a small portion of the leaf (from the same herbarium material) was silica-dried and kept for ca. 2 weeks in plastic bags prior to conducting flow cytometry analyses to estimate the genome size and ploidy level. The specimens are stored in



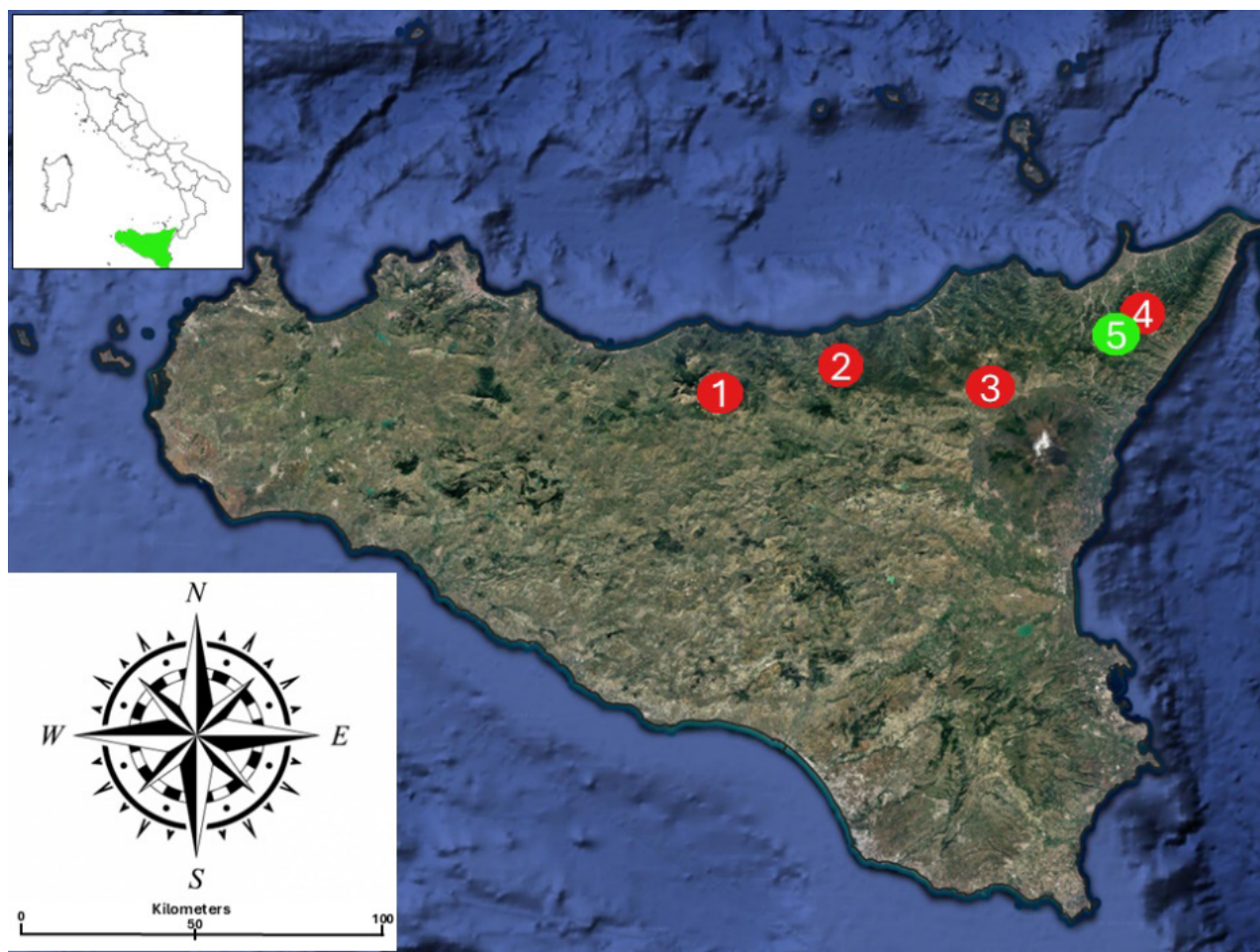


Figure 1. Locations of the records referring to the Sicilian fern populations of *Dryopteris affinis* s.l.

Red, historical records, populations that deserve to be confirmed; Green, extant population reported in this study. 1, Castelbuono (Madonie Mountains, Province of Palermo), including three localities that are very close to each other, i.e., San Guglielmo, Contrada Rosselli and Canalicchio; 2, Caronia (Nebrodi Mountains, Province of Messina); 3, Cannata (Nebrodi Mts., Province of Messina); 4, Santa Lucia del Mela (Peloritani Mountains, Province of Messina); 5, Cataolo (Peloritani Mts., Province of Messina).

the unified Herbarium of the University of Zurich (Z) and in the herbarium of ETH Zurich (ZT), Switzerland.

### Flow cytometry analysis

Silica gel-dried leaves were sent to Plant Cytometry Services (Didam, The Netherlands, [www.plantcytometry.nl](http://www.plantcytometry.nl)) to estimate the genome size through propidium iodide (PI) staining according to the procedure of Bourge *et al.* (2018) and to estimate the ploidy level of the plants. Approximately 1 cm<sup>2</sup> of the fronds of *D. affinis* s.l. samples were mixed with 1 cm<sup>2</sup> of fresh leaves of standard plants (*Clivia miniata*, genome size  $2c = 35.77$  pg of DNA and *Ophioglossum planiscapus*, genome size  $2c = 11.87$  pg of DNA). This mixture was chopped with a sharp razor blade and placed in 100 µl of CyStain nuclei extraction buffer to release the nuclei (Sysmex, Norderstedt, Germany). The obtained suspension was sieved through a 40 µm filter, and 1.5 ml of CyStain PI (propidium iodide) absolute P staining buffer was added. After one hour, the fluorescence of the nuclei in the suspension was measured using a Sysmex ploidy analyzer (Sysmex, Norderstedt, Germany). Each individual was analyzed 1 to 4 times. The ploidy level was estimated based on comparison against all previously published  $2c$

values and chromosome counts (e.g., Ekrt *et al.*, 2009; Šmarda *et al.*, 2019; Zonneveld, 2019).

### Results

#### Morphological identification

Two of the seven investigated individuals were identified as belonging to the diploid *D. affinis* subsp. *affinis* var. *affinis* (individuals A1 and A2, Appendix 1 and 2), while the remaining 5 individuals belonged to triploid *D. robusta* (B1-B5, Appendix 1 and 2) (Fraser-Jenkins, 2007; Freigang & Zenner, 2007). This information will not be lost if future investigations (including genetic studies) confirm the distinctness of *D. robusta*. Within the *D. affinis* group, both taxa often occupy extreme positions in the spectrum of their respective characteristics. At first glance, the leaf blade of *D. affinis* subsp. *affinis* var. *affinis* stands out due to its orderly appearance of a well-formed, funnel-shaped growth pattern (Figure 2, Appendix 2), whereas *D. robusta* has a rather orderless appearance (Figure 3, Appendix 2). The different habits of the two taxa are caused by several characteristic features (Fraser-Jenkins, 2007; Freigang & Zenner, 2007) and explained in detail below.



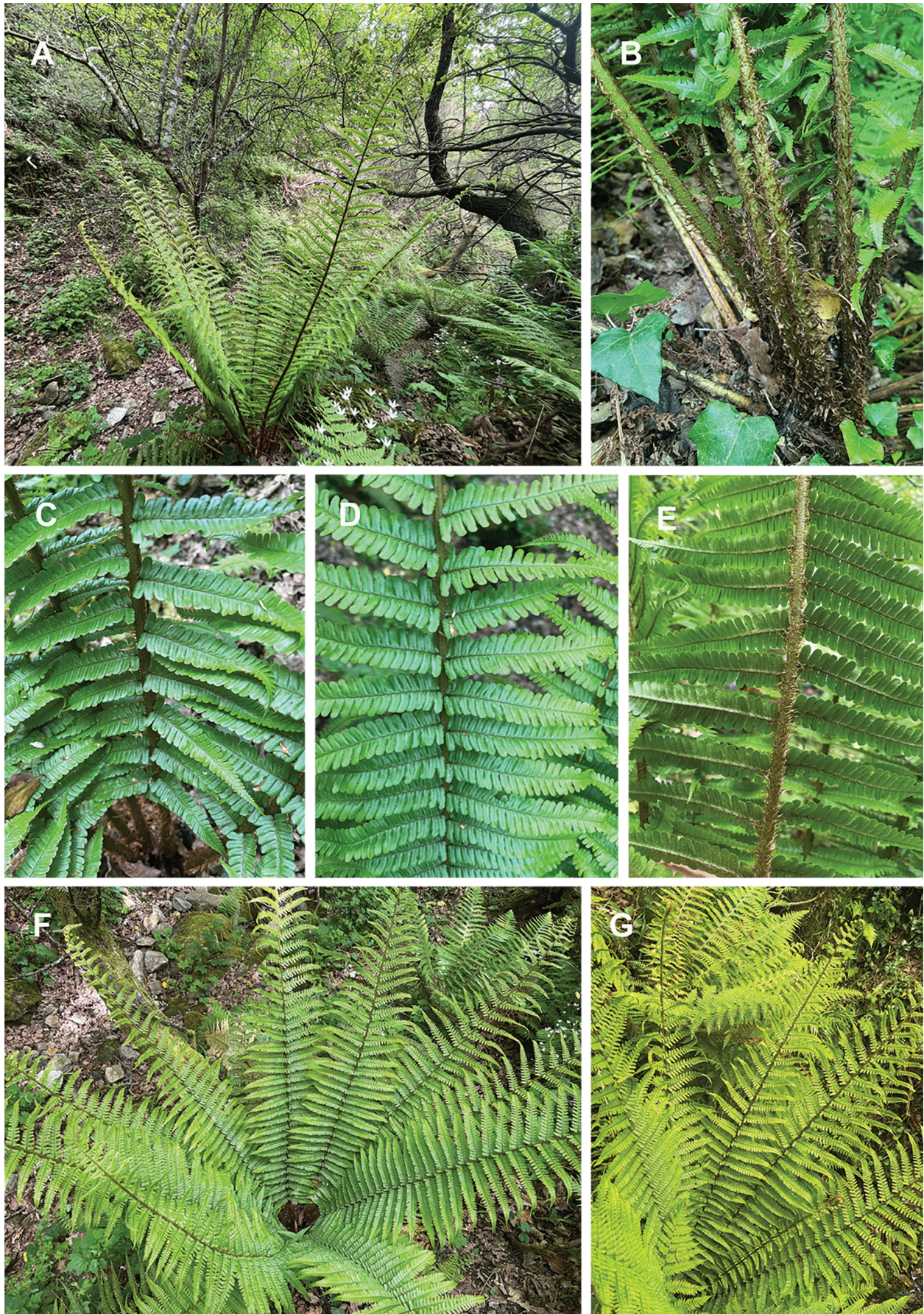


Figure 2. Diploid *Dryopteris affinis* subsp. *affinis* var. *affinis* from Vallone Cataolo. A, Habit of individual A1; B, Stipe scales (A2); C, Frond bases (A1); D, Upper blade of the frond (A1); E, Lower blade of the frond (A1); F, Fronds forming a funnel (A1); G, Fronds of the individual A2.



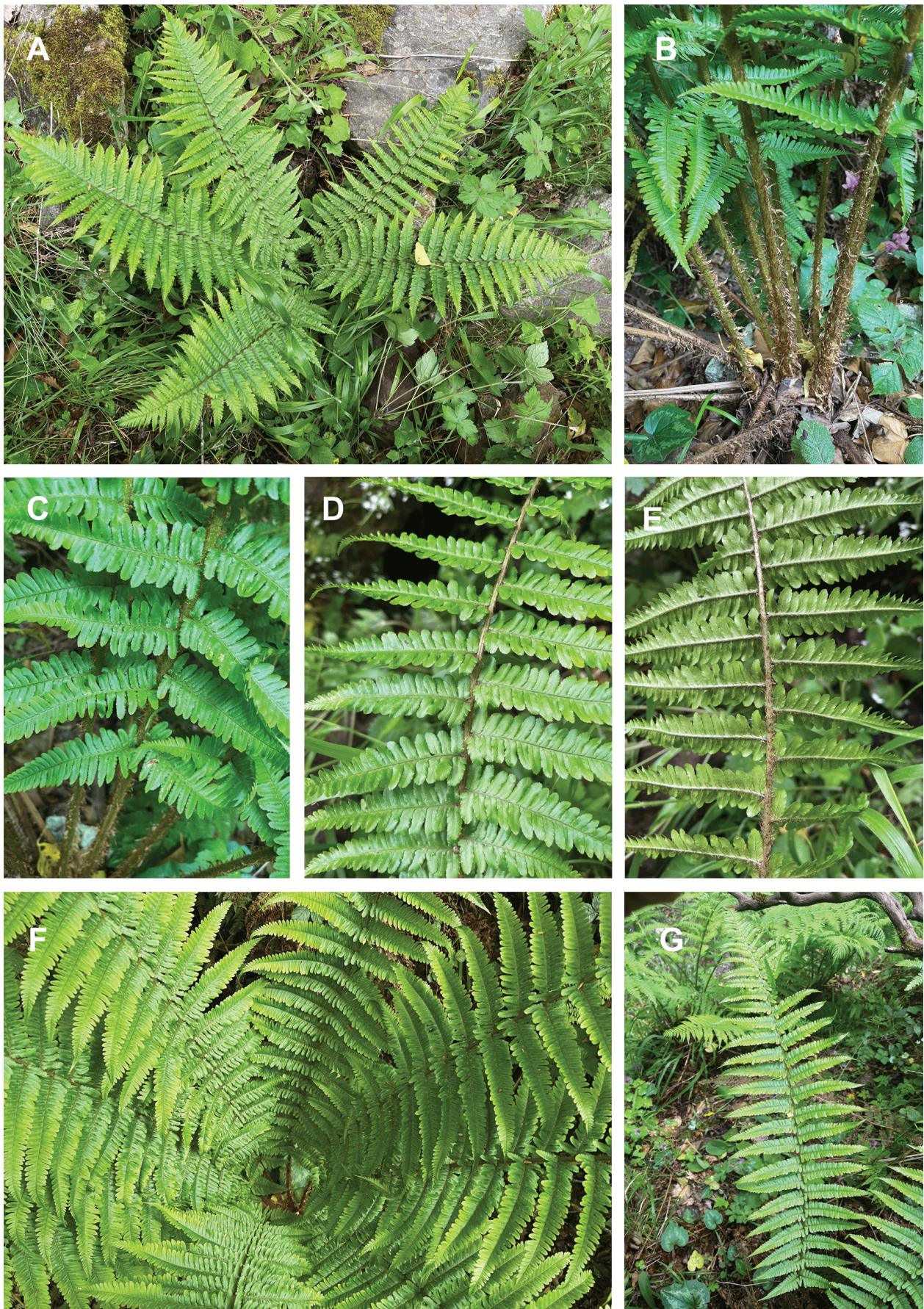


Figure 3. Triploid *Dryopteris robusta* from Vallone Cataolo. A, Habit of individual B3; B, Stipe scales (B1); C, Frond bases (B1); D, Upper blade of the frond (B3); E, Lower blade of the frond (B3); F, Fronds forming a funnel (B1); G, Frond of the individual B4.



**Dryopteris affinis subsp. affinis var. affinis**

In this taxon, the pinnae in the upper half of the leaf are arranged quite evenly in one plane (Figure 2F, Appendix 2), and, in the same plane, the pinnules are also arranged evenly, showing at most a slight curvature at their lateral margin. The rather orderly appearance is emphasized by a uniform and continuous shortening of the pinnules towards the distal end of the pinna (Figure 2D). The leaf shape shows a very uniform arrangement of all plant segments (fronds, pinnae and pinnules) with precisely coordinated length ratios, uniform (mostly U-shaped) curves of the ends of the pinnules and regularly arranged teeth (Figure 2F, G). The high gloss of the leaf blade, its leathery texture (Figure 2C, D) and the strong, dense scaling of the stout petiole (Figure 2B) complete the sturdy habit. The leaf rachis possesses shiny chaff scales (Figure 2E), which tend to be aligned in the same direction and usually have a deep brown to reddish-brown color. After spore maturity, the indusia shrink only slightly due to their leathery consistency; the shape resembles a small dome and only rarely do they tear at the edge.

**Dryopteris robusta**

The appearance of *D. robusta* (Figure 3, Appendix 2) is quite different. Its habit is orderless, coarse and unadorned. Usually, the fronds of the plant are not arranged in an even funnel (Figure 3A); rather, they give the impression that they have already been disheveled by the wind. When looking at the leaf blade in a typical individual, a resting point (in plants with a strong expression of this characteristic) is difficult to find: the pinnae and pinnules appear unordered, the pinnules are unevenly undulated and twisted in all directions, and neighboring pinnules do not display equal torsion (Figure 3A, D, E). The arrangement of the

teeth varies from pinnulae to pinnulae, and the lengths of neighboring pinnules vary so much that sometimes shorter, sometimes longer pinnules stand next to each other in a disordered sequence (Figure 3G). However, the pinnules on the pinnae located in the lower half of the leaf appear more uniform in terms of curvature and section, especially in large, funnel-forming individuals (Figure 3F). In this plane, the pinnules are roughly truncated apically, as if quickly trimmed to the same length with scissors. The leaf blade is dull and matt, its appearance is thin, and the indusia show a parchment-like structure after sporulation, which shrinks into deformed funnel-shaped structures. The rather weak, thin petiole is covered with a sparse scale (Figure 3B). The often dull, deeper, brown-colored chaff scales of the petiole become pale and rather sparse down the leaf rachis (Figure 3E).

**Genome size and ploidy estimates**

To estimate genome size, flow cytometry was used to unambiguously separate the samples of the *D. affinis* species complex found in Vallone Cataolo into two groups, which were congruent with the morphological identification (Table 1, Figure 4). The samples identified morphologically as *D. affinis* subsp. *affinis* var. *affinis* showed 2C-values ranging between 14.00 and 16.90 pg of DNA (Table 1, Table in Appendix 1 and Figure 4), which supported the published estimates for diploid taxa ( $2n = 2x = 82$ ; Ekrt *et al.*, 2009; Šmarda *et al.*, 2019; Zonneveld, 2019). In contrast, samples identified morphologically as *D. robusta* showed higher 2C-values, ranging between 21.30 and 23.80 pg of DNA (Table 1, Table in Appendix 1 and Figure 4), and matched the triploid taxa of the *D. affinis* complex ( $2n = 3x = 123$ ; Ekrt *et al.*, 2009; Šmarda *et al.*, 2019; Zonneveld, 2019).

Table 1. Estimated genome size and ploidy level (mean value  $\pm$  standard deviation) for the two taxa belonging to the *Dryopteris affinis* group found in Vallone Cataolo. The genome size (2c values) is given in pg of nuclear DNA. The estimated ploidy level is based on comparisons with previously published 2c values and chromosome counts (e.g., Ekrt *et al.*, 2009; Šmarda *et al.*, 2019; Zonneveld, 2019).

Taxon	2c value in pg of DNA (mean $\pm$ SD)	Estimated ploidy level
<i>D. affinis</i> subsp. <i>affinis</i> var. <i>affinis</i>	15.50 ( $\pm$ 1.37)	$2n = 2x = 82$ (diploid)
<i>D. robusta</i>	22.73 ( $\pm$ 0.93)	$2n = 3x = 123$ (triploid)

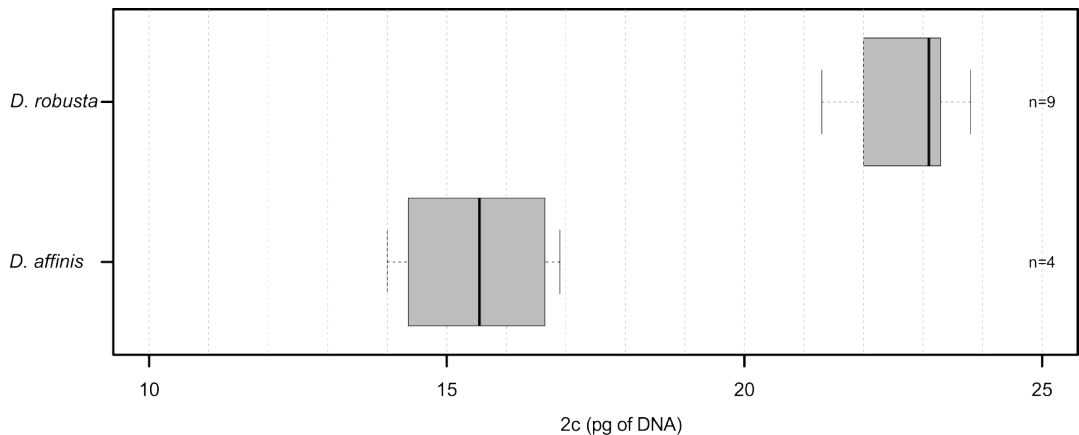


Figure 4. Variation in the genome size of two taxa of the *Dryopteris affinis* group found in Vallone Cataolo. Box plots show the median values, the quartiles and the 5<sup>th</sup> and 95<sup>th</sup> percentiles (whiskers). The number of samples analyzed is indicated on the right of the plot.

## Discussion

The confirmation of *D. affinis* in Sicily and the newly discovered congeneric species, *D. robusta*, underline and support the key role played by the Peloritani Mountains as a refuge for many mesophilous species with a mid-European and boreal range. Considering the position of this mountain range, its role as a refuge is not surprising. Located on the portion of the island that is closest to the Italian Peninsula, this mountain range represents an obligated steppingstone for many species that repeatedly crossed the Strait of Messina during the Pleistocene glaciations. The unexpected find also highlights that, due to their rugged morphology, the Peloritani Mountains are among the least explored areas of the island, with many opportunities for botanical surprises.

Considering the remarkably high humidity of this mountain range (Bazan *et al.*, 2015), the richness of local fern flora, noted by Troia *et al.* (2011), should not be surprising. Most of the large ferns that are of great phytogeographical interest in Sicily, such as *Osmunda regalis* L., *Woodwardia radicans* (L.) Sm. and *Thelypteris palustris* Schott, recently found by Sciandrello *et al.* (2021), occur in this region. Finally, bibliographic and herbarium research focusing on the ferns of the *D. affinis* group highlighted the need to carry out similar work to clarify the past and current presence and distribution of *D. filix-mas* in Sicily. To date, the occurrence of this species on the island has been based exclusively on herbarium specimens from 19<sup>th</sup> century collections kept in LY (Fraser-Jenkins, in Greuter, 1980). Upon closer inspection, the herbarium specimens held in PAL and CAT and previously referred to as *D. affinis* belong to *D. filix-mas* or to *D. pallida* (Bory) Maire et Petitm. subsp. *pallida*, respectively (AC & SP, pers. obs.).

The specimens from the late 19<sup>th</sup> and early 20<sup>th</sup> centuries now attributed to the *D. affinis* species complex suggest a gradual rarefaction of these ferns throughout Sicily, probably due not only to direct damage caused by anthropogenic disturbance but also to global warming. In recent decades, global warming has caused not only an increase in temperatures and a decrease in summer rainfall but also an increase in the frequency of extreme thermal and drought events (Arnone *et al.*, 2013; Pecorino *et al.*, 2024). The same factors threaten many other species that prefer cool and moist environments, such as *Petagnaea gussonei* (Spreng.) Rauschert, *Siculosciadium nebrodense* (Guss.) C. Brullo, Brullo, S.R. Downie & Giusso, *Adenostyles alpina* (L.) Bluff & Fingerh. subsp. *nebrodensis* (Wagenitz & I. Müll.) Greuter and *Woodwardia radicans* (L.) Sm. (Spampinato *et al.*, 2008; De Castro *et al.*, 2013).

Given the small number of surviving populations and the low total number of individuals per stand, it is important to protect and preserve the habitats in which they grow.

## Authorship contribution

GK: conceptualization, research, visualization, writing – review and editing; JF: research, methodology, writing; SP: research, supervision, writing – review and editing;

RG: research, writing; FA: research; LC: data curation, research, resources; GG: research, writing; ASG: writing; CM: writing; RMP: writing; AC: research, writing.

## Conflict of interest

None.

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Appendix 1. Characteristics of investigated individuals of the *Dryopteris affinis* species complex from the Vallone Cataolo.

Taxon / Individuals	Coordinates (N/E)	Altitude (m asl)	2c values (pg of DNA)	Ploidy
<b><i>Dryopteris affinis</i> subsp. <i>affinis</i> var. <i>affinis</i></b>				
A1	38°02'26"/15°15'39"	1100	16.90, 14.00	diploid
A2	38°02'25"/15°15'38"	1120	16.40, 14.70	diploid
<b><i>Dryopteris robusta</i></b>				
B1	38°02'32"/15°15'39"	1070	23.10	triploid
B2	38°02'32"/15°15'39"	1070	23.50	triploid
B3	38°02'31"/15°15'38"	1080	23.30, 23.80, 21.30	triploid
B4	38°02'32"/15°15'38"	1070	22.00	triploid
B5	38°02'32"/15°15'39"	1070	21.40, 22.90	triploid

Appendix 2. Identification key for *Dryopteris affinis* group in Italy

General notes

This identification key is intended exclusively for field use, as many diagnostic characteristics – such as pinnule undulation, frond sheen, surface texture, and the coloration of the rachis – are often no longer discernible in herbarium specimens. The key is suitable only for fertile fronds between 60 cm and 110 cm in length. In larger fronds, features such as the degree of pinnule dentation tend to increase, while in smaller fronds, dentation and scaling (on the rachis and pinna axes) are often less developed or reduced. Only taxa that are taxonomically valid and confirmed to occur in Italy are included in the key. Excluded are *Dryopteris filix-mas* and hybrids between members of the *D. affinis* aggregate and *D. filix-mas* or *D. oreades*. Occasionally, individuals or entire populations of the *D. affinis* group may be encountered in Italy that are not accounted for in the currently accepted taxonomic framework. These unclassified morphotypes are not covered by the key. Many diagnostic traits become apparent only through comparative analysis, which presupposes

a certain level of experience in fern identification. Determination is facilitated by examining multiple fronds from the same plant or from different individuals within a population. However, sympatric occurrence of multiple taxa within the *D. affinis* complex may occur within a single population.

Identification key

- 1 Underside of the rachis bears small, translucent glandular hairs (approx. 50 µm; visible only under a dissecting microscope), present in approx. 90% of individuals of this taxon – the only member of the *D. affinis* aggregate in Central Europe with glands; rachis barely visible in oblique view due to very short petiolules and basal basiscopic pinnules overlapping the rachis.....***D. cambrensis* subsp. *insubrica***
- 1' No glands on the underside of the rachis; rachis clearly visible in oblique view.....2
- 2 Scale covering dense and appearing closed, covering the rachis extensively; numerous scales narrowly lanceolate to filiform; the



delicate scales on the rachillae of the pinnae segments are also mostly distinctly brown in color. Some scales in the middle portion of the rachis exceed 8 mm in length; blade leathery in texture and typically strongly glossy .....3

- 2' Scale covering sparse, loose, not appearing closed, with the rachis only lightly covered; most scales arise from a broad base, shortly extended, not distinctly lanceolate or filiform; the delicate scales on the rachillae of the pinnule segments are at most pale brown to straw-colored (except in *D. lacunosa*). No scales in the middle portion of the rachis are longer than 8 mm; blade soft in texture and usually without a prominent sheen. Indusium of the sori at spore maturity soft, thin, membranaceous, contracting in a funnel-shaped manner, not radially torn; in addition to *D. cambrensis* subsp. *insubrica*, other triploid lineages with  $3n = 123$  chromosomes exist (cytological or flow cytometric confirmation required) .....5
- 3 Upper surface of fertile pinnules (second-order pinnae) with distinct round depressions corresponding to sori on the underside; bidentate teeth on lateral margins of pinnules; middle portion of the blade regularly undulate due to evenly twisted pinnules .....  
***D. affinis* subsp. *punctata***
- 3' Upper surface of fertile pinnules with elongate, comma-shaped depressions at sori locations; no bidentate teeth on lateral margins; blade overall flat and even .....4
- 4 Pinnules narrow, tapering markedly from base, with wide gaps between them (blade resembles

*Blechnum spicant*); blade appears light and semi-transparent, like an open Venetian blind; petiole scales very dense, reddish-brown, sickle-shaped (parallel edges converging only in the apical quarter); rachis scales in middle blade region dense and mostly narrowly lanceolate.....***D. affinis* subsp. *affinis* var. *disjuncta***

- 4' Pinnules broad, with parallel sides tapering only in the upper quarter, resulting in densely spaced pinnae without obvious gaps; blade appears opaque, not light (like a closed Venetian blind); petiole scales dark to reddish brown, with margins that converge gradually; rachis scales in the middle of the blade sparser and mostly hair-like ..... ***D. affinis* subsp. *affinis* var. *affinis***
- 5 Scales on the rachis with a dark brown central stripe, giving a dark appearance; interspaces between pinnules (up to at least the 10<sup>th</sup> pair) strongly boot-shaped, similar to *D. filix-mas*; bidentate teeth present on lateral margins of pinnules ..... ***D. lacunosa***
- 5' Rachis scales straw-colored to light reddish-brown; interspaces between pinnules boot-shaped only up to about the 5<sup>th</sup> pair at most; no (or only occasionally) bidentate teeth on lateral pinnule margins .....6
- 6 Blade surface appears irregularly undulate to crisped, overall dense and opaque, not light (like a closed Venetian blind).....***D. robusta***
- 6' Blade surface appears flat and not crisped, blade relatively open and translucent, light (like an open Venetian blind) ..... ***D. borrieri***