

Equisetum × *moorei* Newman (Equisetaceae) – a ‘new’ nothotaxon in the Polish flora

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Abstract: To investigate present and historical distribution of *Equisetum* × *moorei* in Poland and its habitat requirements, field studies at sites of potential occurrence of this hybrid taxon as well as literature and herbarium search were performed. As a results of these investigations, *E.* × *moorei* was found at five contemporary and at a few historical localities in the present territory of Poland. Since the *Equisetum* populations near Olkusz (S Poland) showed phenotype similar, to some extent, to triploid hybrid *E.* × *ascendens*, we performed nuclear DNA content analysis of these populations. However, it turned out that the investigated individuals belonged to a diploid taxon that can be ascribed to *E.* × *moorei*.

Key words: Central Europe, distribution, *Equisetum*, flow cytometry, hybrid taxon

1. Introduction

Our knowledge of hybrid taxa in the *Equisetum* genus is insufficient, both in Poland and in other European countries. In Poland, information on the occurrence of the following *Equisetum* hybrids: *E.* × *litorale* Kühlew. ex Rupr. (*E. arvense* × *E. fluviatile*), *E.* × *font-queri* Rothm. (*E. palustre* × *E. telmateia*), *E.* × *robertsii* T. D. Dines (*E. arvense* × *E. telmateia*) and *E.* × *trachyodon* (A. Br.) W. D. J. Koch (*E. hyemale* × *E. variegatum*) was recently provided (Wróbel 2013a, 2013b), although the occurrence of *E.* × *robertsii* in Poland requires verification. *E.* × *trachyodon* is the only hybrid taxon within the *Hippochaete* subgenus found in Poland until now (Wróbel 2013b).

Besides these nothotaxa, *E.* × *moorei* Newman (*E. ramosissimum* × *E. hyemale*, syn. *E. hyemale* var. *schleicheri* Milde) was mentioned as a component of Polish flora in the past (Milde 1858; Schube 1903; Decker 1912). However, these reports were not confirmed afterwards (Dostál 1984) and this taxon was never listed as a component of the Polish flora (Mirek *et al.* 2002).

The hybrid between *E. ramosissimum* and *E. hyemale* was described as a separate taxon by E. Newman (1854), based on the material collected in Ireland by a local botanist David Moore, curator of the botanical garden in Dublin. *E.* × *moorei* is one of six hybrid taxa within the *Hippochaete* subgenus found in Europe, besides *E.* × *trachyodon* (A. Br.) W. D. J. Koch (*E. hyemale* × *E. variegatum*), *E.* × *meridionale* (Milde) Chiov. (*E. ramosissimum* × *E. variegatum*), *E.* × *alsaticum* (H. P. Fuchs & Geissert) Philippi (*E. hyemale* × *E. variegatum* × *E. hyemale*), *E.* × *ascendens* Lubienski & Bennert (*E. hyemale* × *E. ramosissimum* × *E. hyemale*) and *E.* × *geissertii* Lubienski & Bennert (*E. hyemale* × *E. ramosissimum* × *E. variegatum*) (Lubienski 2011). The three latter taxa are newly described triploid hybrids, reported so far only from Germany, France and the Netherlands (Lubienski *et al.* 2010, 2012; de Winter & Lubienski 2012).

General distribution of *E.* × *moorei* is still insufficiently known. It is generally given from West and Central Europe. Even though the taxon is rather widespread, it is relatively seldom reported, which is certainly due to difficulties in its identification. In Western Europe,



Fig. 1. Leaf sheaths of *Equisetum hyemale* (left), *E. ×moorei* (middle) and *E. ramosissimum* (right)

the taxon was reported so far from the British Isles (SE Ireland and from Surrey, England, as an ephemerophyte) (Stace 2010; Jepson *et al.* 2013), France (Dubois-Tylski & Girerd 1986; Dardaine & Parent 1997; Prelli 2001; Tison & de Foucault 2014), Spain and Portugal (Prada 1986), Germany (Lubienski 2011), Belgium (Bizot & Pétrement 2010), the Netherlands (de Winter & Lubienski 2012), Luxembourg (Pétrement *et al.* 2012), as well as from north and central Italy (Prelli 2001; Marchetti 2008). In Central Europe, it was reported from the Czech Republic (Hrouda 1997; Kubát *et al.* 2002), Slovakia (Futák 1966) and Hungary (Simon 2000). In the southern part of the continent, *E. ×moorei* occurs in Romania (Grințescu 1952) and Bulgaria (Hájek *et al.* 2005). To the north, the taxon reaches sea coasts of Latvia and Estonia (Laasimer *et al.* 1993) and Swedish Gotland (Mossberg & Stenberg 2003). The latter site is historical (Lubienski, pers. comm.). Information on the isolated localities in the east of the continent (the Volga region, Russia), and even further east in Asia (Kazakhstan) (e.g. Milde 1865; Hrouda 1997) was not confirmed by herbarium specimens (Skvortsov 2008). Among countries where *E. ×moorei* occurs, Poland was also mentioned but no further information was given (e.g. Prelli 2001). In the Polish literature, this taxon has not been so far reported either.

According to literature (Dostál 1984), the phenotype of *E. ×moorei* is intermediate between the parental species, although very variable, deviating often towards one of the parents. The most apparent diagnostic characters are those of leaf sheaths (Stace 2010) (preferably observed in the middle of the shoots): in *E. ×moorei* the length/width ratio is 2 or more (up to 2.3) with a narrow black stripe at the base (Fig. 1), for *E. hyemale* – the

ratio is ca. 1 (up to 1.5) with the broad stripe, while for *E. ramosissimum* – the ratio is ca. 3, the sheaths are bell-shaped without the black stripe at the base (Fig. 1). Another, microscopic diagnostic feature is that of warts on the shoot ribs: in the case of *E. hyemale*, the warts are arranged in 2 rows, while in *E. ramosissimum*, the warts of neighbouring rows are fused to form perpendicular slats (Haeupler & Muer 2007). *E. hyemale* is totally wintergreen, *E. ramosissimum* is not, while the hybrid is mostly not completely wintergreen (Lubienski 2011).

To investigate historical and present distribution of *E. ×moorei* in Poland and its habitat requirements, literature and herbarium search was performed, and field studies at sites of potential occurrence of this hybrid taxon were carried out. As the result of these investigations, *E. ×moorei* was found at five contemporary and at a few historical sites in the territory of Poland.

2. Material and methods

Field studies were conducted during the years 2005–2015. Herbarium search was performed in selected Polish and German herbaria: KRA, KRAM, KTU, LBL, LOD, TRN, WA, WRSL and MSTR (Münster, Germany). Geographical coordinates of the locations were determined with a Garmin Legend HCx and Spectra Precision Mobile Mapper® 10 GPS receivers. The plants were documented using Nikon Coolpix P500 and Olympus XZ-1 cameras. A distribution map in the ATPOL grid (10 × 10 km squares) was prepared using Gnomon 3.3 software.

Plants used for the 2C measurements were obtained from the following sites: *E. ×moorei* – NW of Bolesław

near Olkusz, *E. hyemale* – Kobylańska valley near Kraków, *E. ramosissimum* – Piekary near Kraków. For nuclear DNA content estimation, nuclei were released simultaneously from shoots of a sample species and an internal standard – *Allium cepa* ‘Alice’, 34.89 pg/2C (Doležel *et al.* 1998) by chopping in a Petri dish in 1 ml of nucleus-isolation buffer (0.1 M Tris–HCl, 2.5 mM MgCl₂·6H₂O, 85 mM NaCl, 0.1%, v/v, Triton X-100; pH 7.0), supplemented with propidium iodide (PI; 50 µg cm⁻³) and ribonuclease A (50 µg·cm⁻³). The suspension was passed through a 50-µm mesh nylon filter and analysed using a CyFlow SL (Partec GmbH, Münster, Germany) flow cytometer equipped with a high-grade solid-state laser with green light emission at 532 nm, long-pass filter RG 590 E, DM 560 A, as well as with side (SSC) and forward (FSC) scatters. Analyses were performed on 7-10 individuals of each taxon. For each sample, DNA content was established in 3000-5000 nuclei. Histograms were analyzed using a FloMax (Partec GmbH, Münster, Germany) software. Coefficient of variation (CV) of the G₀/G₁ peak of *Equisetum* sp. ranged between 4.04 and 5.82%. Nuclear DNA content was calculated using linear relationship between the ratio of the 2C peak positions *Equisetum*/*Allium* on a histogram of fluorescence intensities.

The nomenclature of vascular plants, *Equisetum* hybrids and lichens was according to Mirek *et al.*

(2002), Lubienski (2011) and Fałtynowicz (2003), respectively.

3. Results

An atypical population of *Equisetum* sp. was found in 2005 in the valley of the middle Bug river, NW of Góry village, Korczew commune, Mazowieckie province (FD07). Originally, it was supposed that the population consisted of atypical individuals of *E. hyemale*. However, further morphological and phenological research (the individuals were only partially wintergreen) led to the conclusion that it represented *E. ×moorei*. This conclusion was also supported by indirect data, i.e. occurrence of both parental species in the neighbourhood. *E. hyemale* occurs frequently in the Bug river valley and the neighbouring hills (Zajac & Zajac 2001). The species takes/occupies mostly humid habitats, often in oak-hornbeam forests. In such conditions, *E. hyemale* grows, for example, in forests W of Mężenin (5.75 km from the *E. ×moorei* site) and in Bartków (9 km). On the other hand, *E. hyemale* was also found at dry places, on dunes over the Bug river and in pine forests. These sites were located much closer to that of *E. ×moorei* – in Drohicznyn-Topolina (2.5 km), Zajęczniki-Kozerówka (4.25 km) and in Leonów forest in the proximity of Drażniew village (2.5 km). In these cases, the habitat



Fig. 2. Habitat of *Equisetum ×moorei* in Góry (photo by P. Kalinowski, April 2013)

of *E. hyemale* was like that of *E. ×moorei*. Both taxa were found on loose, neat riverside sands at very dry and sunny places. The only, maybe significant, difference was the occurrence of *E. ×moorei* in the secondary, regenerating former agricultural land (Fig. 2), while *E. hyemale* was found in more or less natural habitats, although also affected by anthropopressure. It is worth mentioning that *E. hyemale* was reported from similar, sandy habitats in Belarus, where it is regarded as a rare species (Parfenov 2009). Besides these natural stands, *E. hyemale* in the proximity of *E. ×moorei* occurred also at anthropogenic habitats, like railway embankments in Platerów (12.25 km) and Niemojki (12.50 km).

The other of the parental species of *E. ×moorei* – *E. ramosissimum* – is a rare taxon in Poland, reported relatively frequently only over middle course of the Vistula river (Zajac & Zajac 2001). Furthermore, it occurs rarely in Lublin Upland, in Małopolska and Wielkopolska regions and only exceptionally in other parts of Poland (Zajac & Zajac 2001). One locality was reported from the lower Bug valley in Wyszaków (Ciosek 1976, unpublished). In the ATPOL database, there are no data from the middle Bug valley, although the species was reported by Głowacki (1984) from the same Góry village where *E. ×moorei* occurs. It is probable that this information refers to one of the two currently existing subpopulations of *E. ramosissimum* in the vicinities of Góry. One of them (small, a few square meters) takes side of a sandy field road, S of the village, and partially enters the neighbouring fields (in 2013 these were maize fields). The other (NW of the village, 600 m apart from the former) is only slightly bigger and occupies the sandy top of a small, flat hill together with *E. ×moorei*.

In 2005, when *E. ×moorei* was found in Góry, it took a few square meters at the edge of a young pine forest in close proximity to a crop cultivation. After the cultivation was abandoned (ca. 2008–2010), part of the land was forested and the other part remained fallow land. Afterwards, fast spreading of the hybrid was observed in the young forest and in the uncultivated area. Within a few years, the area covered by *E. ×moorei* increased to ca. 6 100 m². The expansion is especially clearly observed in N direction, on the fallow surrounded by a young pine forest. On the other hand, expansion of *E. ramosissimum* is not observed at this site. Its small sheets are evidently dominated by the hybrid and are difficult to find.

Since phytosociological data are scarce for *E. ×moorei* in Europe, we present 3 phytosociological relevés from the vicinity of Góry:

Relevé 1: N 52° 21' 54.5", E 22° 39' 58.5", C – 70%, D – 40%, 5 × 5 m (inclination 0°),

Agrostis capillaris 1, *Convolvulus arvensis* +, *Conyza canadensis* 1, *Corynephorus canescens* 1, *Elymus*

repens 2, *Equisetum ×moorei* 2, *Hieracium pilosella* 4, *Jasione montana* 1, *Filago minima* 1, *Setaria pumila* +, *Cladonia rei* 3, *Cladonia maclineata* 1
Relevé 2: N 52° 21' 55.7", E 22° 39' 55.1", C – 85%, D – 40%, 5 × 5 m (inclination 0°),

Artemisia campestris 1, *Conyza canadensis* 2, *Corynephorus canescens* 3, *Elymus repens* 1, *Equisetum ×moorei* 3, *Festuca rubra* 1, *Hieracium pilosella* 2, *Holcus lanatus* 1, *Jasione montana* 2, *Pinus sylvestris* +, *Setaria pumila* +, *Solidago virga-aurea* 1, *Viola arvensis* +, *Cladonia fimbriata* +, *Cladonia rei* 2, *Racomitrium canescens* 1

Relevé 3: N 52° 21' 54.6", E 22° 39' 52.1", C – 90%, D – 0%, 5 × 5 m (inclination 0°),

Achillea millefolium 2, *Agrostis capillaris* 1, *Alopecurus pratensis* +, *Calamagrostis epigeios* +, *Convolvulus arvensis* 1, *Conyza canadensis* 1, *Elymus repens* 1, *Equisetum ×moorei* 2, *Equisetum ramosissimum* 2, *Festuca rubra* 3, *Hieracium pilosella* 3, *Hypochoeris radicata* +, *Jasione montana* 1, *Knautia arvensis* 1, *Oenothera* sp. +, *Peucedanum oreoselinum* 1, *Poa pratensis* 1, *Pyrus communis* +, *Rumex acetosella* 1, *Saponaria officinalis* 1, *Solidago virga-aurea* 2, *Taraxacum* sp. +, *Viola arvensis* +

In the south of Poland, *E. ×moorei* was found in 2013 along one draining ditch (Dąbrówka canal) and edges of the neighbouring pine forest, NE of Bolesław near Olkusz (N 50° 18' 10.1", E 19° 29' 51.0") (Małopolska province, DF36). The locality is situated close to the Pomorzany zinc-lead mine. The population covered an area of at least 200 m² and was composed of erect and creeping individuals reaching often 1 m or more in height, mostly wintergreen (Fig. 3). The leaf sheaths of some of the individuals looked different depending on the season of the year. During wintertime, they appeared typical for *E. ×moorei* (Fig. 3), while during summertime, they resembled those of *E. ramosissimum* to some extent. There were no parental species found together with the hybrid. Nevertheless, the literature data (Zajac *et al.* 2006) indicate close proximity of *E. hyemale* and the nearest locality of *E. ramosissimum* is ca. 6 km apart. However, these sites were not confirmed in the present study.

Since the individuals of *E. ×moorei* found in the vicinity of Bolesław showed some similarity in phenotype to the triploid *E. ×ascendens* (tall, totally wintergreen shoots) reported by Lubiński (2011), we performed flow cytometric analysis of nuclear DNA content (2C) of the hybrid and the parental species (Table 1). The obtained data showed that all the investigated populations had similar 2C DNA (about 54 pg), corresponding to diploid taxa: *E. hyemale* 52.6 pg/2C and *E. ramosissimum* 56.4 pg/2C (Bennert *et al.* 2005). This confirmed that the investigated population was diploid and excluded the possibility that it was represented by *E. ×ascendens*.

Table 1. Nuclear DNA content of *Equisetum* cf. *×moorei*, *E. hyemale* and *E. ramosissimum*. All specimens were collected in the vicinity of Kraków

Taxon	DNA content (pg/2C ± SD)
<i>Equisetum</i> cf. <i>×moorei</i>	54.78 ± 0.35
<i>Equisetum hyemale</i>	53.36 ± 0.72
<i>Equisetum ramosissimum</i>	54.56 ± 0.56

In 1998, *E. ×moorei* was found by Marcus Lubienski (pers. comm.) in Kartuzy surroundings near Gdańsk, SW of Przodkowo (CA88). It occurred numerously in the area of a sand pit, north of the Kartuzy-Wejherowo road. The photograph of the hybrid from this site was included in one of the articles of the founder (Lubienski *et al.* 2012), however, this finding was not confirmed formally. It is documented by two herbarium sheets provided by M. Lubienski (presently in the private collection of J. Kruk). However, during our field studies in 2015, the presence of *E. ×moorei* was not confirmed at this site.

Revision of the collections of the selected herbaria resulted in the following findings of *E. ×moorei*:

KTU: DF46, Bukowno, railway station, track-ways, leg. & det. T. Nowak, 28.08.1992, KTU079638 and 079639 (ut *E. hyemale*);

LBL: FE32, Kępa Chotecka near Opole Lubelskie, river dyke, leg. & det. D. Fijałkowski, 3.09.1963, 779 (ut *E. hyemale* f. *viride*); FD77, Borki near Radzyń, pine-oak forest, leg. & det. D. Fijałkowski, 14.09.1965, 770, 773 & 774 (ut *E. hyemale* f. *genuinum*);

LOD: FD92, Borowa (Puławy district), humid sand-bank on the Vistula river, leg. & det. Dobrzeńska-Osiak B., 6.09.2001, LOD152159 (ut *E. ramosissimum*);

TRN: DA81, by the sea behind the Vistula river estuary, leg. E.F. Klinsmann, 1851 (ut *E. hyemale* var. *schleicheri*); CB65, Zdręczno Lake, Tuchola district, leg. M. Ceynowa, 25.07.1968 (ut *E. hyemale*);

WA: ED16, Warsaw, slope of a dyke close to Myśluborska street, leg. E. Korcz, 23.07.1978, WA4081 (ut *E. hyemale*) – in 2015, the hybrid was not confirmed at this locality because of strong anthropogenic changes of the Vistula river banks in this area.

WRSŁ: BE49, Wrocław-Karłowice, on the old Odra river, leg. R. Uechtritz, 09.1853, WRSŁ000385 (ut *E. hyemale schleicheri*) – among *E. ramosissimum* on the



Fig. 3. *Equisetum ×moorei* near Bolesław (photo by J. Kruk, February 2015)

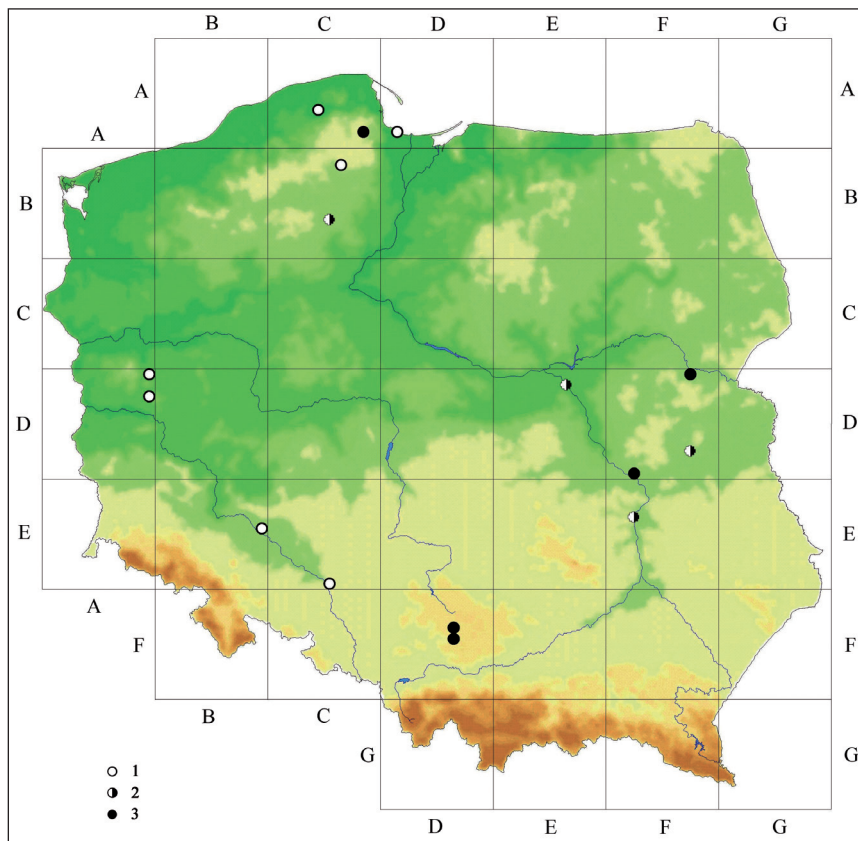


Fig. 4. Distribution of *Equisetum ×moorei* in Poland, in the ATPOL grid, based on the literature, herbarium records and present findings
 Explanations: 1 – historical localities before 1945, 2 – localities from the period 1945-1990, 3 – contemporary localities (after 1990)

same sheet; BE49, Wrocław, leg. R. Uechtritz, 09.1853, WRSL000375 (ut *E. hyemale schleicheri*); BE49, Wrocław-Karłowice, not on sands of the old Odra river but in dense *Prunus spinosa*-bushes at the Odra dyke, leg. C. G. Baenitz, 15.10.1894, WRSL000376 (ut *E. hyemale schleicheri*)

MSTR: (all sheets ut *E. hyemale*, rev. M. Lubiński); BE49, near Wrocław, leg. P. Heuser, 18??, 143552; DA81, Gdańsk: ad mare balticum, Möwenschanze, leg. C. G. Baenitz, 7.08.1889, 143466 and 143476; BE49, Wrocław-Karłowice, at the Odra dyke, leg. C. G. Baenitz, 20.10.1896; BE49, Wrocław, leg. C. G. Baenitz, 15.10.1894, 143411 and 143441; DA81, Gdańsk: ad mare balticum, leg. C. G. Baenitz, 15.07.1877, 143435; BE49, Wrocław, leg. C. G. Baenitz, 11.07.1895, 143409 and 143412.

Revision of the historical literature (Schube 1903; Decker 1912), referring to the present territory of Poland, revealed the following sites of occurrence of *E. ×moorei* (present Polish names of the localities are given): Chociule near Świebodzin: towards Łąkie (AD29); Jordanowo: road to Nowy Dworek in a pine forest, frequently (AD09); Wrocław-Dąbie, Wrocław-Karłowice, Wrocław-Biskupin (all BE49); Opole-Zakrzów (behind Moritzberg) (CE95). The taxon was

also reported as *E. hyemale* var. *schleicheri* from a range of Silesian and Pomeranian localities by Milde (1858), most of which, however, were secondary citations after other German scholars (Caspary, Klinsmann, Nolte, von Uechtritz) and were mostly not confirmed afterwards: Kościerzyna (CB16), Gdańsk (DA81), Lębork (CA64) and Opole (CE95). It seems that only the locality in Wrocław-Karłowice was known to Milde himself (well before Schube's report), but all of them are fully reliable.

4. Discussion

The present finding of *E. ×moorei* in the territory of Poland extends the area of its occurrence in Europe.

The site in Góry is very interesting since it allows reconstruction of probable mechanisms of the nothotaxon genesis, existence and spreading. The stand is a rare example of the occurrence of *E. ×moorei* together with both parental species, although not directly with *E. hyemale*. Distribution of the nothotaxon in Europe is only partially due to the occurrence of its parental species. Many researchers emphasize its independent distribution of one or both parents and this fact is well documented at many sites in Europe, from classical Irish

populations (natural stands of *E. ramosissimum* in the British Islands are doubtful) (Jepson *et al.* 2013) to a recently described locality in Luxembourg (Pétrement *et al.* 2012). Also in Central Europe, this hybrid, at least at some stands, occurs independently of both parents or, more frequently, only together with *E. ramosissimum* (Hrouda 1997). In the case of the Bug riverside locality, the key factor that led to the hybrid formation could have been the adaptation of local populations of *E. hyemale* to rather extreme conditions for this species, dominating on sandy dunes. In this way, habitat and spatial boundaries between both parental species of *E. ×moorei* were eliminated. Broad distribution of *E. ×moorei* in Europe is explained by its ability to reproduce vegetatively, even from small rhizome fragments. This could favour spreading and existence of this taxon in areas where one or both parental species do not occur – in Ireland and in the Baltic region, for example (Lubienski 2011). This reproduction mechanism is also observed in Góry, because it is the only reproduction strategy for *E. ×moorei*, since – as a hybrid taxon – it forms only sterile spores. Until now, however, no distant spreading of this taxon in the proximity of Góry has been observed.

Information on habitats where *E. ×moorei* was found in Europe are rather general. According to Dostál (1984), typical habitats for this nothotaxon are sandy,

open forests, sandy ‘semi-steppes’, dunes, meadows, etc. According to Hrouda (1997), it grows in habitats similar to those of *E. ramosissimum* (also in secondary ones), such as sands, pine forests, sandy fields, road and railway embankments. In Italy, it was found on sands and in ditches (Marchetti 2008), in an old gravel pit in Luxembourg (Pétrement *et al.* 2012), in dry, fresh forests and on sea coasts in Estonia and Latvia (Kull *et al.* 2002; www.latvijasdaba.lv). *E. ×moorei* occurs also on sea shores and dunes in Ireland (Stace 2010).

In Poland, *E. ×moorei* was found in a sand pit (Lubienski, pers. comm.), on river dykes and sea shores, as well as in a former agricultural land of the *Corynephorion canescentis* associations, on the borders of young pine forests, along draining ditches and on edges of pine forests.

The presented data indicate that *E. ×moorei* was, and continues to be a rare nothotaxon dispersed throughout most of the Polish territory but it is probably more widespread than the described localities indicate.

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