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ORIGINAL PAPER

Invasion of *Eragrostis albensis* in Central Europe: distribution patterns, taxonomy and phylogenetic insight into the *Eragrostis pilosa* complex

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Abstract The *Eragrostis pilosa* complex (Poaceae) comprises five widely distributed and regionally invasive species—*E. albensis*, *E. amurensis*, *E. imberbis*, *E. multicaulis*, and *E. pilosa*, distinguished by tiny and variable morphological characters and with so far unknown phylogenetic relationships. Recently, some doubts have been raised about the status of an invasive glandular morphotype occurring in Central Europe assigned either to *E. amurensis* or to *E. albensis*. Here, we addressed this issue by analysing morphology, internal transcribed spacers of nuclear ribosomal DNA, and five inter-simple sequence repeat markers. The genetic evidence supported closer relationship of this glandular morphotype to eglandular *E. albensis*, widely established in Central Europe, than to glandular *E. amurensis* described from Asia. We propose to adopt a new taxonomic treatment that *E. albensis* includes both eglandular and glandular individuals, and to classify the glandular ones as *E. albensis* var. *scholziana* M. Nobis & A. Wróbel *var. nova.* Currently this new taxon is known from a dozen of localities in Central Europe and is invasive in the lower section of the Oder River valley, whereas *Eragrostis albensis* var. *albensis* has already spread widely across Europe in riparian phytocenoses and anthropogenic habitats. Since probably the first registered records in 1940s, it has been observed in European part of Russia, Belarus, Ukraine, Poland, Slovakia, Czech Republic, Germany, Austria, the Netherlands, and its further invasion is likely to proceed. We provided distribution maps concerning spread dynamics of *E. albensis* in Europe from 1947 to 2020. In total, the species has been observed on over 1300 localities so far, most of which were found after 2000.

Keywords Alien invasive species · Cryptic invasion · Distribution · *Eragrostis albensis* var. *scholziana* · Integrative taxonomy · Lovegrasses

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Introduction

Biological invasions are currently considered one of the major global concerns threatening biodiversity and triggering economic losses (Pejchar and Mooney 2009; Pyšek and Richardson 2010). To prevent or at least mitigate such undesirable effects, immediate and precise identification of spreading newcomers is necessary in order to take appropriate countermeasures. Many alien species, however, belong to taxonomically problematic groups which makes their detection challenging and, therefore, usually time-delayed (Verloove 2010; Pyšek et al. 2013). Such cryptic invaders could be easily misidentified due to their morphological similarity to other closely related taxa and may remain unnoticed in the field for a long time. Moreover, insufficient knowledge about distribution of these organisms as well as routes and time of their spread may lead to difficulties while classifying them either as native or introduced in a particular region (Saltonstall 2002; Pyšek et al. 2013; Morais and Reichard 2017).

Nowadays, however, development and application of molecular markers may help to avoid confusion between invaders and other closely related native taxa, particularly those of conservation interest, as well as with other non-native taxa or species of economic importance (e.g. Newmaster and Ragupathy 2009; Cseke and Talley 2012; Wong et al. 2018; Martinez et al. 2020). The molecular approach can also be successfully applied to unravelling the population genetic structure of some invasive taxa, determining their routes of spread and sites of origin, locating their traces in environmental DNA, examining of their interactions and impact on native organisms, as well as identifying the features driving invasion success (e.g. Briski et al. 2011; Meyer et al. 2016; Hardouin et al. 2018; Fagúndez and Lema 2019). All of these issues are particularly relevant to biodiversity management and essential for the effective allocation of efforts and funding (Pyšek et al. 2013).

Genetic-based tools are thus appropriate candidates for application to taxonomically problematic groups such as grasses (Poaceae), a large (ca 12,000 species; Watson and Dallwitz 1992) monocot family of hard-to-identify plants occurring on all continents. Many grasses have been purposely propagated worldwide due to their agricultural and ornamental properties, while others have expanded their ranges spontaneously (David and Baruch 2000; Maillet and Lopez-Garcia 2000; Canavan et al. 2019). As a consequence, grasses include many examples of problematic invasive species, such as representatives of the following genera: *Arundo*, *Bromus*, *Echinochloa*, *Eragrostis*, *Imperata*, *Pennisetum*, *Phalaris*, *Phragmites*, *Spartina* (CABI 2020; Global Invasive Species Database 2020).

One of the largest groups of grasses is *Eragrostis* Wolf (lovegrasses), which comprises ca 400 species (Clayton et al. 2006) and is globally distributed from tropical to temperate regions, being mainly associated with dry, sandy, or human-disturbed areas. Therefore, some *Eragrostis* taxa are widely used as crop plants and forage (e.g. *E. tef* (Zucc.) Trotter; Cheng et al. 2017) or for erosion control (e.g. *E. curvula* (Schrad.) Nees; Lee et al. 2013), and have been intentionally introduced into many regions worldwide, where they have spread within both natural and anthropogenic habitats (Muranaka and Washitani 2004; Guzik and Sudnik-Wójcikowska 2005; Michalewska and Nobis 2005; Pagitz 2012). As a result, in many countries, the representatives of *Eragrostis* are now classified as alien casual or naturalised species, and regionally regarded as invasive (e.g. Pyšek et al. 2009; Yoshioka et al. 2010; Tokarska-Guzik et al. 2012).

In Central Europe, occurrence of *Eragrostis* could be dated back at least to the first half of the XIX century when, probably for the first time, *E. minor* Host was found in Silesia in 1838 within current borders of Wrocław in Poland (Fiek 1881). Since that time, over a dozen annual alien *Eragrostis* taxa have been observed in Central Europe and some of them have already become naturalised in this area (Špryňar and Kubát 2004; Guzik and Sudnik-Wójcikowska 2005; Scholz and Ristow 2005; Király et al. 2011; Medvecká et al. 2012; Hohla 2013). On a regional scale, two taxa are considered invasive species—*E. minor* in the Czech Republic (Danihelka et al. 2012) and *E. albensis* H. Scholz in Poland (Tokarska-Guzik et al. 2012; Dajdok et al. 2018). Other species, such as *E. multicaulis* Steud. and *E. pilosa* (L.) P. Beauv., are also spreading in Central Europe but have not been classified yet as invasive in this region (Scholz and Ristow 2005; Hohla 2006).

Recently, the occurrence of another species, *E. amurensis* Prob., was recorded in Central Europe along the Oder River valley in Germany in 2003 (B herbarium; Scholz and Ristow 2005) and in Poland in

2005 (WRSL, KRA herbarium; Kącki and Szczęśniak 2009) as well as along the Inn River valley in Upper Austria in 2013 (LI herbarium; Hohla 2013). *Eragrostis amurensis* was described as a new species from the Amur Province, Russian Far East on the basis of presence of numerous glands on a whole plant, especially on leaf sheaths, unlike mostly eglandular *E. pilosa* (Probatova and Sokolovskaya 1981). *Eragrostis amurensis* (= *E. voronensis* H. Scholz) as well as *E. albensis*, *E. imberbis* (Franch.) Prob., *E. multicaulis*, and *E. pilosa* belong to taxonomically problematic and widely distributed *E. pilosa* complex (Seregin 2012a). The most common characters used in the identification of these species are small differences in the morphology of the panicle and spikelets as well as presence or absence of glands and hairs on cauline leaf sheaths (Probatova 1985; Špryňar and Kubát 2004; Seregin 2012a). After delimitation of *E. amurensis* as a new species (Probatova and Sokolovskaya 1981), revision of herbarium materials and observations of its spread in the field have demonstrated that it occurs in a vast area of temperate Russia, Mongolia, Kazakhstan (Seregin 2012a), Tajikistan (Nobis et al. 2015; Wróbel et al. 2017), Belarus, Ukraine (Seregin 2012a; Parfenov 2013), and in Central Europe (Scholz and Ristow 2005; Kącki and Szczęśniak 2009; Hohla 2013).

However, Pagitz (2012) and Hohla (2013) have recently expressed some doubts about taxonomic treatment of the glandular *E. amurensis* specimens observed in Austria. Pagitz (2012) inclined to the view that glandular specimens from the North Tyrolean population found in 2005 (IB herbarium) could rather belong to *E. albensis* even though this species at that time was accepted as eglandular (Scholz 1995; Scholz and Ristow 2005). Pagitz (2012) suggested that *E. albensis* could probably include both eglandular and glandular morphotypes and put forward such a hypothesis for further studies. Hohla (2013) finally followed the taxonomic approach of Seregin (2012a) and classified the glandular plants from the Inn River valley in Upper Austria as *E. amurensis*, however, he highlighted their probable close relationship with the glandular specimens from North Tyrol. During our preliminary research focused on morphology of *E. albensis* and its spread in Poland, we observed that glandular plants from the Oder River valley, so far the only other known glandular population from Central Europe and previously also assigned to *E. amurensis* (Scholz and Ristow 2005; Kącki and Szczęśniak 2009), are extremely similar to eglandular *E. albensis* occurring in this region. Thus, our findings were in line with the previous speculations of Pagitz (2012) and Hohla (2013).

Eragrostis albensis differs from *E. pilosa* mostly in having shorter spikelet pedicels, slightly longer lemmas and glumes, more prominent lemma veins, usually no hairs at the apex of upper leaf sheaths, more scabrid and stiff panicle branches, and no verticillate branches in the lowest panicle node (Sholz 1995; Špryňar and Kubát 2004; Nobis and Nobis 2009). *Eragrostis albensis* was proposed to be regarded as a Central European young endemic taxon by Scholz (1995), at that time with known localities only from the Elbe and the Oder River valleys. Later, the revision of herbarium materials demonstrated that the occurrence of *E. albensis* in Germany could be dated back to 1982 when it was collected in Berlin (Scholz and Ristow 2005), while along the Elbe River valley the species had been documented since 1991 (Scholz and Ristow 2005), and from the Oder River valley since 1992 (Scholz 1995). Soon after, many other localities of *E. albensis* were reported from Central and Eastern Europe owing to the revision of herbaria material and new field observations. Probably the oldest collected specimen of *E. albensis* in the region is dated back to 1947 when the species was discovered along the Vistula River valley within current borders of Warsaw, Poland (WA herbarium; Guzik and Sudnik-Wójcikowska 2005). In 1968, *E. albensis* was for the first time found in Slovakia along the Danube River in Bratislava (Portal 2002) and in the same year in the Czech Republic in Prague (PR herbarium; Špryňar and Kubát 2004). These records are over 10 years older than the first findings in Germany dated back to 1982 (Scholz and Ristow 2005). Due to such pattern of distribution, Špryňar and Kubát (2004) proposed that *E. albensis* could rather be an invader in Central Europe, probably originating in other, more eastern parts of Eurasia. Nevertheless, the exact location of the origin spot of *E. albensis* was not determined. In Eastern Europe, where less research has been devoted to *E. albensis*, the first registered records of the species could probably be dated back to 1975 when it was collected in Bryansk, European part of Russia (MW herbarium; Seregin 2012b); later the species was also confirmed in Ukraine in 1997 (herbarium in Kiev; Gubar 2004) and Belarus (Guzik

and Sudnik-Wójcikowska 2005). So far, the species was also found in Austria (Hohla 2006; Pagitz 2012) and the Netherlands (L herbarium). Despite increasing evidence about history of *E. albensis* spread, the exact location of its native range remains undetermined and a question whether it is autochtonous to Central Europe has not been fully answered yet (Špryňar and Kubát 2004; Pagitz 2012; Hohla 2013). Despite such circumstances, currently the leading approach is to recognize *E. albensis* in Central Europe as the alien invader (Špryňar and Kubát 2004).

An ongoing discussion about taxonomic treatment of Central European glandular *Eragrostis* specimens, so far either assigned to *E. amurensis*, *E. amurensis*-like plants or to *E. albensis*, has raised a question about their phylogenetic relationship as well as the current distribution and morphological variability of both *E. amurensis* and *E. albensis* (Scholz and Ristow 2005; Pagitz 2012; Hohla 2013). Due to such uncertainties and the observed invasive character of this glandular *Eragrostis* morphotype in Central Europe, the accurate identification of these grasses is particularly relevant for better understanding differentiation and routes of spread of the taxa within the *E. pilosa* complex which is essential for biodiversity management. Despite the growing interest in studies of *Eragrostis* (e.g. Cannarozzi et al. 2014; Carballo et al. 2019; Somaratne et al. 2019), molecular evidence concerning variability and relationships between the taxa from the *E. pilosa* complex still remains insufficient. This study aims to clarify the status of the glandular Central European *Eragrostis* morphotype from the complex by means of molecular markers. Due to the observed morphological character of these glandular plants as well as their occurrence in the close vicinity of eglandular *E. albensis*, we hypothesise that they are more closely related to eglandular *E. albensis* than to glandular *E. amurensis* and, therefore, that *E. albensis* comprises both eglandular and glandular morphotypes. To our knowledge, this research constitutes the first molecular phylogenetic insight into the *E. pilosa* complex with implications for its taxonomy and provides the most recent summary concerning spread of *E. albensis* in Central Europe.

Materials and methods

In this study we focused on five taxa from the *E. pilosa* complex (*E. albensis*, *E. amurensis*, *E. imberbis*, *E. multicaulis*, *E. pilosa* as well as Central European glandular *Eragrostis* specimens so far either assigned to *E. amurensis* or *E. albensis*). We also added five other *Eragrostis* taxa occurring in Eurasia: *E. cilianensis* (All.) Vignolo ex Janch., *E. minor*, *E. pectinacea* (Michx.) Nees, *E. suaveolens* A. K. Becker ex Claus, and *E. virescens* J. Presl. Extensive herbarium material was reviewed in order to determine morphological patterns among the examined *Eragrostis* taxa and to identify the most informative characters for the taxa from the *E. pilosa* complex. In total, more than 1500 specimens of *Eragrostis* were examined (materials deposited in the herbaria KRA, KRAM, LE, LI, SZUB, WA, WRSL and the herbarium of the University of Opole; acronyms according to Thiers 2020). Representative specimens were chosen for the morphological and molecular part of the research. For taxonomic comparisons, specimens of *E. amurensis* were purposely selected from the species′ core distribution area in Asia (specimens from Russian Siberia and Tajikistan).

Morphology

All examined specimens selected for morphological analyses were listed in "Online Appendix 1". Measurements of panicles, spikelets, upper glumes, lower glumes, lemmas, and caryopses were carried out together with analyses of presence of glands, hairs, and prickles on different parts of culms (Online Appendix 2). Correlation between quantitative variables was checked using the Pearson correlation in RStudio version 1.1.423 (RStudio Team 2016) with R ver. 3.6.1 (R Core Team 2019). Variables characterised by a strong correlation coefficient (more than 0.90 or less than − 0.90) were then individually assessed; one of each pair was excluded from numerical analysis, since applying both might have influenced the final clustering arrangement unfavourably. Finally, the 20 most informative quantitative and qualitative characters (Online Appendix 2) were taken into consideration. The distance matrix was calculated by means of Gower′s similarity index using the *proxy* package in R (Meyer and Buchta 2019). The UPGMA (unweighted pair group method with arithmetic mean)

cluster analysis was performed in R using the *stats* package (R Core Team 2019) to detect morphological patterns among the examined plants.

Molecular analyses

DNA from dried leaf tissue of herbarium specimens was isolated using a Genomic Mini AX Plant Spin (A&A Biotechnology, Poland) kit in accordance with the manufacturer's protocol. Where necessary, the isolated DNA was purified using a gDNA Clean kit (Syngen, Poland). The purity and concentration of extracted DNA was assessed using a NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific, USA). Only pure DNA with an A260/280 (DNA: protein ratio) value larger than 1.8 was used for the downstream analyses.

For ITS analysis we chose 25 individuals (Online Appendix 3). ITS1-5.8 S-ITS2 as a whole was amplified using forward primer N18L18: 5′-AAGTCGTAACAAGGTTTC-3′ (Wen and Zimmer 1996) and reverse primer ITS4: 5′-TCCTCCGCTTATTGATATGC-3′ (White et al. 1990). Amplification reactions were performed in a total volume of 25 µl, containing 10 ng of genomic DNA, 1 × final concentration of PCR DreamTaq Green Buffer (Thermo Scientific, USA), 1 U of DreamTaq Green DNA Polymerase (Thermo Scientific, USA), 0.12 mmol of dNTPs (Thermo Scientific, USA), 0.2 pmol of each primer, and 1 µg of bovine serum albumin (BSA). The PCR mixtures were heated at 94 °C for 3 min prior to 25–30 cycles of PCR amplification in a Veriti thermal cycler (Applied Biosystems, USA); one PCR cycle consisted of denaturation at 94 °C for 1 min, annealing of primers at 50 °C for 2 min, and extension at 72 °C for 2 min; following the last cycle, the PCR mixtures were incubated at 72 °C for 7 min. Reactions without DNA were used as negative controls. PCR products were sent to an external company (Genomed, Poland) for paired-end Sanger sequencing. The resulting sequences were manually verified and aligned using BioEdit ver. 7.0.5.3 (Hall 1999). One sequence of *Eragrostis pectinacea* was taken from GenBank (accession number: GU359301.1) and added to the dataset. One sequence of *Enneapogon desvauxii* (GenBank accession number: GU359339.1) was used as an outgroup.

As no studies had been published on the use of chloroplast markers for the *E. pilosa* complex, here we tested four cpDNA regions widely used in phylogenetic studies of angiosperms. The *trnK-matK* intron was amplified using primers trnK-3914F: 5′-TGG GTT GCT AAC TCA ATG G-3′ (Johnson and Soltis 1994) and matK-AR: 5′-CTG TTG ATA CAT TCG A-3′ (Osaloo et al. 1999). The trnC^{GCA}-*rpoB* intergenic spacer was amplified using primers trnC^{GCA}R: 5′-CAC CCR GAT TYG AAC TGG GG-3′ and rpoB: 5′-CKA CAA AAY CCY TCR AAT TG-3′, modified by Shaw et al. (2005) after Ohsako and Ohnishi (2000). The *petL-psbE* intergenic spacer was amplified using primers petL: 5′-AGT AGA AAA CCG AAA TAA CTA GTT A-3′ and psbE: 5′-TAT CGA ATA CTG GTA ATA ATA TCA GC-3′ (Shaw et al. 2007). The *rpl32-trnL*^{UAG} intergenic spacer was amplified using primers trnL^{UAG}: 5′-CTG CTT CCT AAG AGC AGC GT-3′ and rpL32-F: 5′-CAG TTC CAA AAA AAC GTA CTT C-3′ (Shaw et al. 2007). As a preliminary screening, selected specimens, representing *E. albensis*, *E. amurensis*, *E. multicaulis*, *E. pilosa*, and the glandular *Eragrostis* morphotype from Poland, were used to check the usefulness of selected cpDNA for genetic delimitation of species from the *E. pilosa* complex. All four cpDNA loci were amplified in a total volume of 25 µl, containing 10 ng of genomic DNA, 1 × final concentration of PCR DreamTaq Green Buffer (Thermo Scientific, USA), 1 U of DreamTaq Green DNA Polymerase (Thermo Scientific, USA), 0.12 mmol of dNTPs (Thermo Scientific, USA), 0.08 pmol of each primer, and 2 µg of BSA. The PCR mixtures were then heated at 80 °C for 5 min prior to 35 cycles of PCR amplification in a Mastercycler DNA thermal cycler (Eppendorf, Germany); one PCR cycle consisted of denaturation at 94 °C for 1 min, annealing of primers at 46 °C for 1 min, and extension at 72 °C for 2 min; following the last cycle, the PCR mixtures were incubated at 72 °C for 5 min. Reactions without DNA were used as negative controls. PCR products were sent to an external company (Genomed, Poland) for paired-end Sanger sequencing.

For analyses of ISSR (inter-simple sequence repeat; Ziętkiewicz et al. 1994) markers, 14 samples of *Eragrostis* were chosen (Online Appendix 3, Table 4). During a preliminary stage, the usefulness of 12 selected primers, including six previously designed for *E. tef* (Assefa et al. 2003), was assessed. Finally, five

reproducible and scorable primers with polymorphic bands were chosen and used in PCR reactions in optimised annealing conditions (Table 1).

The PCR amplification of DNA fragments using the five ISSR primers was carried out in a total volume of 15 µl, containing 10 ng of genomic DNA, 1 × final concentration of PCR DreamTaq Green Buffer (for primers 811, 888, 889; Thermo Scientific, USA) or Taq Buffer with $(NH_4)_2SO_4$ (for primers ubc836 and M2; Thermo Scientific, USA), 0.375 U (for 811, 888, 889) or 1 U (for ubc836) or 0.75 U (for M2) of DreamTaq Green DNA Polymerase (Thermo Scientific, USA), 3 pmol of dNTPs (Thermo Scientific, USA), 1.33 pmol of primer, 30 pmol of $MgCl_2$ (Thermo Scientific, USA) for 811, 888, 889, and ubc836 or 45 pmol for M2.

The PCR mixtures were heated at 95 °C for 3 min prior to 33 cycles of PCR amplification in a DNA thermal cycler T100 (Bio-Rad, USA); one PCR cycle consisted of denaturation at 95 °C for 30 s, annealing of a primer at 53–58 °C (Table 1) for 30 s, and extension at 72 °C for 1 min; following the last cycle, the PCR mixtures were incubated at 72 °C for 10 min. For each primer, the PCR reaction was repeated to check reproducibility.

ISSR fragments were separated via electrophoresis for one hour at 100 V in 2% agarose gel with Midori Green DNA stain (Nippon Genetics, Germany) for visualisation. 1X TBE was used as a buffer solution. Subsequent imaging was carried out under UV light to confirm the amplification. The size of amplified fragments was estimated via comparison to a GeneRuler 100 bp Plus DNA Ladder (Thermo Scientific, USA) and scored in a binary format as either (1) present or (0) absent.

Molecular data analyses

Maximum-likelihood analysis (ML) of ITS marker was performed in PhyML 3.0 (Guindon et al. 2010). The best substitution model for the dataset was determined using SMS (Smart Model Selection; Lefort et al. 2017) with AIC (Akaike Information Criterion). The model GTR + I (general time-reversible model with a proportion of invariable sites) was indicated as most appropriate. Support values for the tree nodes were calculated using the approximate likelihood ratio test (aLRT; Anisimova and Gascuel 2006).

Bayesian Inference (BI) analysis was performed in MrBayes 3 (Ronquist and Huelsenbeck 2003). The most appropriate substitution model was selected using MrModeltest 2.3 (Nylander 2005) and PAUP* 4.0a166 (Swofford 2002). Model SYM + G (a symmetrical model with gamma distributed rate variation among sites) was indicated as the best and was set as lset nst = 6 rates = gamma, prset statefreqpr = fixed(equal). An MCMC simulation was set as a default for 1,000,000 generations, sampling one of every 500 generations, which sufficed to obtain the average standard deviation of split frequencies below 0.01 and the potential scale reduction factor of all parameters close to 1.0. Tracer 1.6.0 (Rambaut et al. 2014) was used to assess convergence and to ascertain whether all statistics were characterised by effective sample sizes (ESS) greater than 200. The first 25% of the iterations were discarded as a 'burn-in' fraction; the remainder was used to construct the Bayesian consensus tree.

The trees were edited in TreeGraph 2 (Stöver and Müller 2010) and in MEGA ver. 7.0.26 (Kumar et al. 2016). Bayesian posterior probabilities (BPPs) ranged from 0.90 to 1 and support values from ML (MLs) higher than 0.7 (Hillis and Bull 1993) were regarded as sufficiently strong support for clades. Tree nodes with weak support, with both BPPs less than 0.9 and MLs less than 0.7, were collapsed and presented as unresolved (polytomy). To increase the clarity of the phylogram, the location of support values for each node was changed from the default tree output and adjusted manually using Inkscape ver. 3.

Table 1 Primers used in ISSR analysis concerning examined *Eragrostis* taxa

Code	Sequence (5′ to 3′)[a]	Length	Annealing temperature (°C)
811	GAG AGA GAG AGA GAG AC	17	53
888	BDB CAC ACA CAC ACA CA	17	53
889	DBD ACA CAC ACA CAC AC	17	58
ubc836	AGA GAG AGA GAG AGA GYA	18	53
M2	ACA CAC ACA CAC ACA CYG	18	53

[a]B = Non-A (i.e. C, G or T); D = Non-C (i.e. A, G or T); Y = Pyrimidine (C or T)

A binary ISSR matrix containing only polymorphic bands was analysed in RStudio version 1.1.423 (RStudio Team 2016) with R ver. 3.5.3 (R Core Team 2019). The distance matrix was calculated by means of the Jaccard similarity index using the *proxy* package (Meyer and Buchta 2019). A neighbour-joining tree and bootstrap analysis were performed using the *ape* package (Paradis and Schliep 2018). Bootstrap values higher than 0.7 were regarded as sufficiently strong support for clades (Hillis and Bull 1993). The tree was rooted by *Eragrostis* taxa from outside the *E. pilosa* complex.

Spread dynamics of Eragrostis albensis

Distribution maps of *E. albensis* spread in Europe were based on the specimens of this taxon preserved in the herbaria KRA, KRAM, LI, POZ, SZUB, WA, WRSL, information in online databases of herbarium specimens of B, BRNU, GJO, LZ, PRC, W, WU (JACQ—Virtual Herbaria; https://www.jacq.org/; accessed 2020-09-10), and MW (https://plant.depo.msu.ru/module/itemsearchpublic; accessed 2020-09-10), published localities (Online Appendix 5), ATPOL database (Zając A., Institute of Botany, Jagiellonian University; accessed 2020-09-14), GBIF database (https://doi.org/10.15468/dl.qc8cf9; accessed 2020-09-04), PLADIAS database (https://pladias.cz/en/; accessed 2020-09-10), observations of Wrzesień M. (Maria Curie-Skłodowska University in Lublin), and unpublished data from our field studies. Original GPS coordinates from a locality were used if possible. If GPS data was not available, geographic coordinates were approximated based on a description of a locality or a number of a grid unit (ATPOL, PLADIAS). Some regions including south-western Czech Republic, south-western Germany, central Poland, Slovakia, Hungary and Eastern Europe could be to some extent underestimated due to scarce or none data available. The list of all *E. albensis* localities which were included in the distribution maps is available in "Online Appendix 6". Spread dynamics of *E. albensis* was divided into three time intervals and set arbitrarily to 1979, 1999, and 2020. The distribution maps were prepared in ArcGIS Desktop 10.8 with the ESRI basemap World Imagery. https://www.arcgis.com/home/item.html?id=10df2279f9684e4a9f6a7f08febac2a9.

Results

Morphology: key characters

Analyses revealed five main distinct morphological groups within the *E. pilosa* complex (Fig. 1; Table 2). Two taxa, *E. multicaulis* and *E. pilosa*, were aggregated. Each formed its own cluster, which was clearly separated from the other taxa (Fig. 1, cluster IV and V, respectively). *Eragrostis amurensis* was linked in one cluster with Central European glandular specimens from the Inn River valley (Fig. 1, cluster I). Central European glandular specimens from the Oder River valley (Fig. 1, cluster III) were resolved as a sister cluster to an aggregation consisting of *E. albensis* and *E. imberbis* (Fig. 1, cluster II).

The most useful diagnostic characters of *E. pilosa* are: the presence of several verticillate branches in the lowest panicle node (in small individuals sometimes non-verticillate), presence of tufts of long hairs at the apex of all leaf sheaths, hairs in the panicle axils, and usually flexuous panicle branches. *Eragrostis amurensis* is very similar to *E. pilosa*, however, it has glands on leaf sheaths and blades. *Eragrostis albensis* has more robust and stiff panicle branches than *E. pilosa*, no verticillate branches in the lowest panicle node (if there are several branches, then they are grouped on one side of a panicle or in clusters opposite to each other, never arranged as a whorl), tufts of long hairs at the apex of usually only lower leaf sheaths, and hairs in panicle axils. *Eragrostis imberbis* is similar to *E. albensis*, however, it has longer pedicels of the lateral spikelets [*E. imberbis*: 2–5 mm long; *E. albensis*: (0.5–)1.0–2.5(–3.5) mm] and has usually several verticillate branches at the lowest panicle node. *Eragrostis multicaulis* has no hairs in panicle axils, no verticillate branches in the lowest panicle node, usually small robust panicle with branches diverging even to 90° at maturity, and no tufts of long hairs at the apex of all leaf sheaths (rarely, at most single long hair on some leaf sheaths).

The analyses showed that, when morphology was considered exclusively, Central European glandular specimens did not represent uniform patterns. Specimens from the Oder River valley referred morphologically to eglandular *E. albensis* to a greater extent than to glandular *E. amurensis* (Fig. 1). These plants had the longest lower glumes within the entire studied complex. Moreover, similarly as in *E. albensis* and *E.*

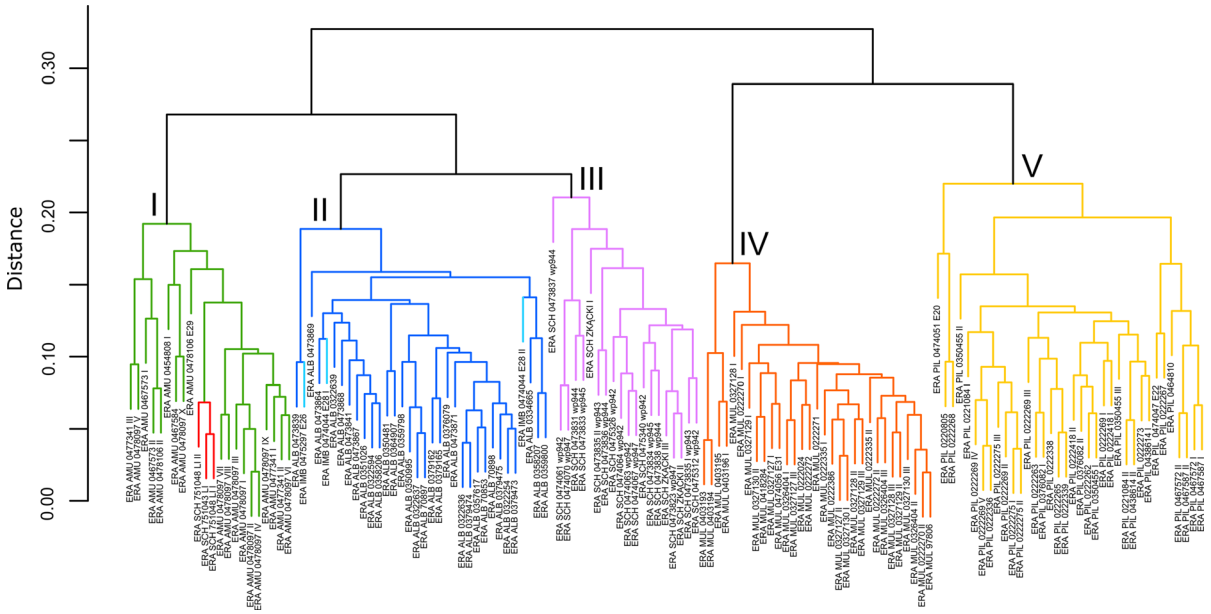

Fig. 1 Dendrogram of the cluster analysis (UPGMA) of the *Eragrostis pilosa* complex based on 20 selected morphological characters (Online Appendix 2) using Gower's similarity index. I—*E. amurensis* (green), glandular *Eragrostis* morphotype from the Inn River valley, Austria (red); II—*E. albensis* (dark blue), *E. imberbis* (light blue); III—glandular *Eragrostis* morphotype from the Oder River valley, Poland (pink); IV—*E. multicaulis* (orange); V—*E. pilosa* (yellow). A list of examined specimens can be found in "Online Appendix 1"

imberbis (Fig. 1; Table 2), they had no tufts of long hairs at the apex of the uppermost leaf sheaths. Specimens from the Inn River valley reflected glandular *E. amurensis* to a greater extent than eglandular *E. albensis* (Fig. 1). These plants had less prominent glumes than specimens from the Oder River valley. In addition, they were characterised in part by long hairs at the apex of the uppermost leaf sheaths (Fig. 1; Table 2). All of the other morphological characters of Central European glandular morphotype overlapped considerably.

ITS region

The ITS sequences numbered 606 bp in all taxa from the *E. pilosa* complex and from 600 to 606 bp in other examined *Eragrostis* taxa. The tree topologies from the Bayesian inference method and the maximum-likelihood analysis based on ITS region were consistent. The Central European glandular *Eragrostis* morphotype was grouped in a clade with eglandular *E. albensis*, *E. imberbis*, and *E. multicaulis* (Fig. 2). The glandular specimens from the Oder River valley were identical with *E. albensis*. They differed, however, from the glandular specimen from the Inn River valley in Upper Austria by two substitutions (Table 3). All representatives of this clade, both glandular and eglandular differed from glandular *E. amurensis* by at least two substitutions (Table 3). In total, six sites were polymorphic in *E. amurensis*, *E. albensis*, *E. imberbis*, *E. multicaulis*, and the glandular morphotype from Central Europe. *Eragrostis virescens* appeared to be a sister clade to *E. pilosa* and other taxa from the *E. pilosa* complex. *Eragrostis minor*, *E. suaveolens*, *E. pectinacea* and *E. cilianensis* subsp. *starosselskyi* were resolved as more distantly related to the *E. pilosa* complex and formed a distinct clade (Fig. 2).

Chloroplast DNA regions

All studied chloroplast regions showed lower level of variation than ITS. Based only on the acquired sequences of good quality, several mutations were detected. In *trnK-matK* intron, one substitution was noted in *E. amurensis* (E2) in comparison to identical sequences of *E. albensis* (E6) and *E. multicaulis* (E17). In *rpoB-trnC*^{GCA} intergenic spacer, two substitutions

Table 2 Comparison of the most informative morphological characters within the *Eragrostis pilosa* complex, based on the specimens listed in "Online Appendix 1"

Character	Taxon						
	E. albensis	*E. amurensis*	*Eragrostis* sp. (glandular; from the Oder River valley, Central Europe: DE, PL)	*Eragrostis* sp. (glandular; from the Inn River valley, Central Europe: AT)	*E. imberbis*	*E. multicaulis*	*E. pilosa*
Tufts of long hairs at the apex of upper leaf sheaths	Absent	Present; rarely absent	Absent	Absent or present	Absent	Absent	Present; rarely absent
Tufts of long hairs at the apex of lower leaf sheaths	Present; very rarely absent but then hairs in the panicle axils present	Present	Present	Present	Present	Absent; rarely with single long hair (not tufts)	Present
Hairs in panicle axils	Present	Present; rarely absent	Present	Present	Present	Absent	Present
Branches at the lowest panicle node	1–2, if more then usually grouped on one side of panicle axis (not verticillate)	1–2, Rarely more verticillate	1–2, If more then usually grouped on one side of panicle axis (not verticillate)	1, Rarely more and partly verticillate	Usually 3 or more verticillate	1–2	Usually 3 or more verticillate; rarely 1–2
Pedicels	Scabrous to densely scabrous	Sparsely to densely scabrous	Scabrous to densely scabrous	Scabrous to densely scabrous	Scabrous to densely scabrous	Glabrous or scabrous only at uppermost pedicels	Usually glabrous to sparsely scabrous; rarely scabrous at uppermost pedicels
Glands on the glumes or lemmas keel	Absent	Present (sometimes)	Absent	Absent	Absent	Absent	Absent
Glands on the leaf sheaths keel	Absent	Present	Present	Present	Absent	Absent	Absent
Glands on leaf blades margins	Absent	Present (sometimes)	Present	Present	Absent	Absent	Absent
Glands below culm nodes (as bands)	Absent	Present (sometimes)	Absent	Absent	Absent	Absent	Absent
Florets in spikelet (number)	(4–)5–8(− 9)	4–7(− 9)	5–9(− 11)	6–8	(5–)6–10	(5–)6–9(− 12)	(4–)5–8(− 11)
Lower glume length (mm)	(0.4–)0.6–1.0	(0.4–)0.5–0.7(− 0.8)	(0.75–)0.9–1.2(− 1.3)	(0.5–)0.6–0.8	0.7–0.9	0.4–0.7(− 0.8)	(0.2–)0.3–0.6(− 0.7)
Upper glume length (mm)	(1.1–)1.2–1.5(− 1.6)	(0.9–)1.0–1.3(− 1.7)	(1.25–)1.3–1.6(− 1.8)	1.2–1.3	1.25–1.4(− 1.5)	(0.8–)0.9–1.2(− 1.3)	(0.6–)0.7–1.1(− 1.4)
Lemma of the lowest floret in the spikelet length (mm)	(1.5–)1.6–1.9(− 2.1)	(1.2–)1.6–1.9(− 2.0)	(1.6–)1.8–2.0(− 2.2)	1.6–1.8(− 1.9)	1.7–1.8(− 2.0)	(1.4–)1.5–1.7(− 1.8)	(1.2–)1.4–1.7(− 1.9)
Lemma of the central floret in the spikelet length (mm)	(1.1–)1.3–1.5(− 1.75)	1.3–1.6(− 1.7)	(1.4–)1.5–1.7(− 1.8)	1.4–1.6	(1.4–)1.5–1.6	(1.0–)1.2–1.4(− 1.5)	(1.1–)1.2–1.4(− 1.5)

Table 2 continued

Character	Taxon						
	E. albensis	*E. amurensis*	*Eragrostis* sp. (glandular; from the Oder River valley, Central Europe: DE, PL)	*Eragrostis* sp. (glandular; from the Inn River valley, Central Europe: AT)	*E. imberbis*	*E. multicaulis*	*E. pilosa*
Length (lower glume / lemma of the lowest floret in the spikelet) ratio	(0.25–)0.3–0.55(– 0.6)	(0.2–)0.3–0.4(– 0.55)	(0.4–)0.5–0.65(– 0.8)	(0.25–)0.35–0.45(– 0.5)	0.4–0.5	0.3–0.45(– 0.5)	(0.15–)0.2–0.45(– 0.5)
Length (lower glume / upper glume) ratio	(0.35–)0.45–0.75(– 0.9)	(0.3–)0.4–0.6(– 0.7)	(0.5–)0.6–0.8(– 0.85)	(0.35–)0.45–0.65	0.55–0.75(– 0.85)	(0.35–)0.4–0.75(– 0.8)	(0.25–)0.4–0.6(– 0.7)

were observed in *E. pilosa* (E22) in comparison to *E. albensis* (E6). In *petL-psbE* intergenic spacer, one insertion was noted in *E. amurensis* (E2) in comparison to glandular morphotype from Central Europe (E34). In *rpl32-trnL* intergenic spacer, one deletion and nine substitutions were observed in *E. pilosa* (E22) in comparison to identical *E. amurensis* (E2), *E. albensis* (E6), *E. multicaulis* (E17), and glandular morphotype from Central Europe (E34).

ISSR markers

In total, the selected set of primers enabled the acquisition of 144 unique bands (characters). ISSR markers indicated that *E. amurensis* differed from the Central European glandular morphotype which was aggregated with eglandular *E. albensis* (Fig. 3; Table 4). *Eragrostis imberbis* appeared to be very closely related to *E. albensis* and the Central European glandular morphotype. *Eragrostis amurensis*, *E. multicaulis*, and *E. pilosa* were considered distinct lineages; however, relationships between these three taxa and *E. albensis* remained unresolved due to weak support (Fig. 3). The other three examined taxa, *E. minor*, *E. virescens*, and *E. cilianensis* subsp. *starosselskyi*, were regarded as distinct lineages outside the *E. pilosa* complex (Fig. 3).

Taxonomic treatment

As a result of morphological and phylogenetic analyses of the *Eragrostis pilosa* complex, we propose to describe the glandular morphotype of *Eragrostis albensis* as follows:

Eragrostis albensis var. *scholziana* M. Nobis & A. Wróbel, *var. nov*. (Online Appendix 4).

TYPE: Western Poland, Lubuskie Province, Słubice, the Oder River valley, sandy banks on the right side of the river, 52°21′4.28″N/14°33′19.32″E, 17 m a.s.l., 26 August 2017, *M. Nobis, A. Nowak, A. Wróbel* s.n. (holotype KRA 474060!; isotypes KRA 474059, 474061–474064, 475309, 475310, 475312, 475313, 475315–475326, 475328, 475329, 475331, 475332, 475334, 475335, 475339–475346, 482318, 528738–528761!).

Diagnosis: *Eragrostis albensis* var. *scholziana* differs from eglandular *E. albensis* var. *albensis* in having glands on leaf sheaths keel and blade margins.

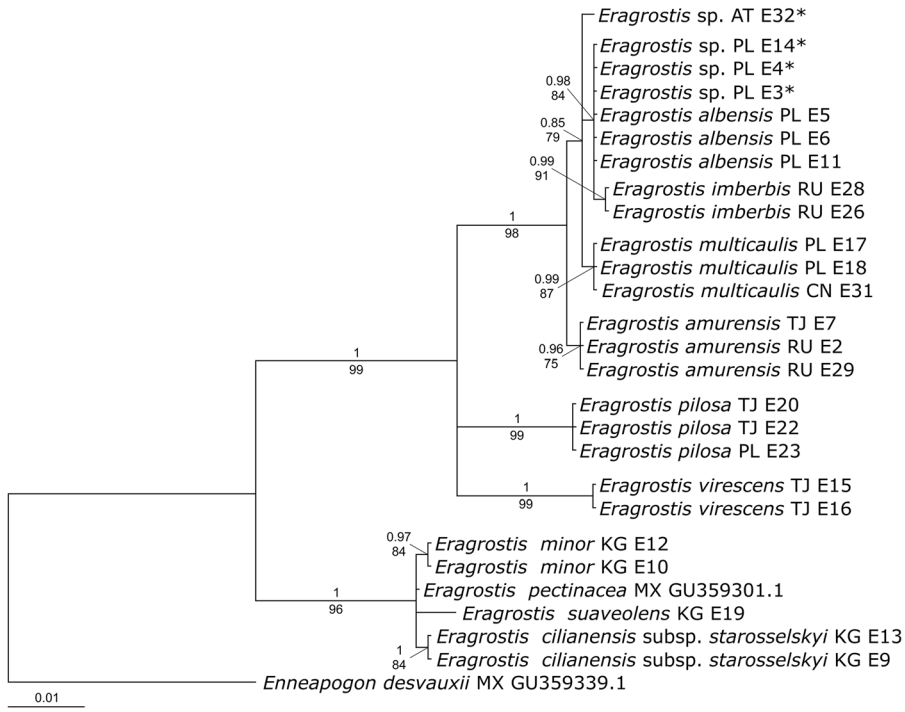

Fig. 2 The phylogram from the Bayesian inference based on ITS1-5.8 S-ITS2 (nuclear ribosomal DNA). Numbers at nodes represent Bayesian posterior probabilities (upper) and ort values from maximum-likelihood analysis (lower). The scale bar represents substitutions per position. Abbreviations: Austria (AT), China (CN), Kyrgyzstan (KG), Mexico (MX), Poland (PL), Russia (RU), Tajikistan (TJ). *Central European glandular morphotype from the *Eragrostis pilosa* complex. All examined specimens are listed in "Online Appendix 3"

Other specimens studied (paratypes): western Poland, Lubuskie Province, Ługi Górzyckie, near the Oder River valley, field road, 52°32′2.27″N/14°37′20.33″E, 14 m a.s.l., 26 August 2017, *M. Nobis, A. Nowak, A. Wróbel* s.n. (KRA 473835, 482319!); western Poland, Lubuskie Province, Ługi Górzyckie, the Oder River valley, sandy banks on the right side of the river, 52°32′8.42″N/14°36′23.99″E, 10 m a.s.l., 26 August 2017, *M. Nobis, A. Nowak, A. Wróbel* s.n. (KRA 473831, 473836–473838, 47473–474075, 474078, 74079, 528718–528721!); north-western Poland, Zachodniopomorskie Province, between Czelin and Stary Błeszyn, the Oder River valley, sandy banks on the right side of the river, 52°44′41.56″N/14°21′32.96″E, 3 m a.s.l., 26 August 2017, *M. Nobis, A. Nowak, A. Wróbel* s.n. (KRA 473833, 473834, 474084, 475307, 475308, 482320!); north-western Poland, Zachodniopomorskie Province, Gozdowice, the Oder River valley, sandy banks on the right side of the river, 52°45′49.94″N/14°19′9.17″E, 5 m a.s.l., 26 August 2017, *M. Nobis, A. Nowak, A. Wróbel* s.n. (KRA 475302–475305, 528711–528717!); north-western Poland, Zachodniopomorskie Province, Osinów Dolny, the Oder River valley, sandy banks on the right side of the river, 52°51′26.94″N/14°8′19.61″E, 1 m a.s.l., 26 August 2017, *M. Nobis, A. Nowak, A. Wróbel* s.n. (KRA 473823, 474067–474070, 474119, 528722–528738!); Kunice, Odra, alluvia, 3 September 2005, *Z. Kącki* s.n. (WRSL!); Porzecze (1), Odra, 3 September 2005, *Z. Kącki* s.n. (WRSL!); Czelin, Odra, alluvia, 2 September 2005, *Z. Kącki* s.n. (WRSL!); Szumiłowo, Odra (3), 3 September 2006, *Z. Kącki* s.n. (WRSL!); Górzyca (1), Odra, alluvia, 3 September 2005, *Z. Kącki* s.n. (WRSL!); Austria, Inn River region, Hagenauer Bucht St. Peter am Hart [Österreich, Oberösterreich, Innviertel, St. Peter am Hart, Hagenauer Bucht, junge Anlandungen an der Südwestseite der "Kellerinsel"], 13°5′47″E/48°16′32″, MTB:7744/2, Unschärferadius: 100 m, 334 m, 15 September 2013, *M. Hohla* (LI 75143!);

Table 3 Polymorphic sites within ITS1-5.8 S-ITS2 (nuclear ribosomal DNA) in examined taxa from the *Eragrostis pilosa* complex vs eglandular *E. albensis* from the Oder River valley, Poland (first row, sample PL E5)

Taxon	Sample	Nucleotide position													
		16	29	51	56	57	76	92	97	112	124	151	178	195	228
E. albensis	PL E5	C	T	A	T	C	T	A	G	A	T	T	G	C	T
E. albensis	PL E6	.	.	.	.	.	.	.	.	.	.	.	.	.	.
E. albensis	PL E11	.	.	.	.	.	.	.	.	.	.	.	.	.	.
E. sp. [a]	PL E14	.	.	.	.	.	.	.	.	.	.	.	.	.	.
E. sp. [a]	PL E3	.	.	.	.	.	.	.	.	.	.	.	.	.	.
E. sp. [a]	PL E4	.	.	.	.	.	.	.	.	.	.	.	.	.	.
E. sp. [a]	AT E32	.	.	.	.	.	.	.	.	G	.	.	.	.	.
E. imberbis	RU E26	.	.	.	.	.	.	.	.	.	.	.	.	.	.
E. imberbis	RU E28	.	.	.	.	.	.	.	.	.	.	.	.	.	.
E. multicaulis	PL E17	.	.	.	.	.	.	.	.	G	.	.	.	.	.
E. multicaulis	PL E18	.	.	.	.	.	.	.	.	G	.	.	.	.	.
E. multicaulis	CN E31	.	.	.	.	.	.	.	.	G	.	.	.	.	.
E. amurensis	TJ E7	.	.	.	.	.	C	.	.	G	.	.	.	.	.
E. amurensis	RU E2	.	.	.	.	.	C	.	.	G	.	.	.	.	.
E. amurensis	RU E29	.	.	.	.	.	C	.	.	G	.	.	.	.	.
E. pilosa	TJ E20	A	C	G	A	T	C	C	T	G	C	C	A	T	C
E. pilosa	TJ E22	A	C	G	A	T	C	C	T	G	C	C	A	T	C
E. pilosa	PL E23	A	C	G	A	T	C	C	T	G	C	C	A	T	C

Taxon	Sample	Nucleotide position													
		350	385	416	420	537	540	541	551	554	555	581	582	586	591
E. albensis	PL E5	G	C	T	C	T	C	C	C	A	C	A	A	C	T
E. albensis	PL E6	.	.	.	.	.	.	.	.	.	.	.	.	.	.
E. albensis	PL E11	.	.	.	.	.	.	.	.	.	.	.	.	.	.
E. sp. [a]	PL E14	.	.	.	.	.	.	.	.	.	.	.	.	.	.
E. sp. [a]	PL E3	.	.	.	.	.	.	.	.	.	.	.	.	.	.
E. sp. [a]	PL E4	.	.	.	.	.	.	.	.	.	.	.	.	.	.
E. sp. [a]	AT E32	.	T	.	.	.	.	.	.	.	.	.	.	.	.
E. imberbis	RU E26	.	.	.	.	.	.	.	.	.	.	.	T	.	.
E. imberbis	RU E28	.	.	.	.	.	.	.	.	.	.	.	T	.	.
E. multicaulis	PL E17	T	.	.	.	.	.	.	.	.	.	.	.	.	.
E. multicaulis	PL E18	T	.	.	.	.	.	.	.	.	.	.	.	.	.
E. multicaulis	CN E31	T	.	.	.	.	.	.	.	.	.	.	.	.	.
E. amurensis	TJ E7	.	.	.	.	.	.	.	.	.	.	.	.	G	.
E. amurensis	RU E2	.	.	.	.	.	.	.	.	.	.	.	.	G	.
E. amurensis	RU E29	.	.	.	.	.	.	.	.	.	.	.	.	G	.
E. pilosa	TJ E20	.	.	C	T	G	T	T	T	G	A	T	.	.	C
E. pilosa	TJ E22	.	.	C	T	G	T	T	T	G	A	T	.	.	C
E. pilosa	PL E23	.	.	C	T	G	T	T	T	G	A	T	.	.	C

The same nucleotide is marked as a dot

Eragrostis (*E.*); country: Austria (AT), China (CN), Kyrgyzstan (KG), Poland (PL), Russia (RU), Tajikistan (TJ)

[a]Central European glandular morphotype from the *E. pilosa* complex. All examined specimens are listed in "Online Appendix 3"

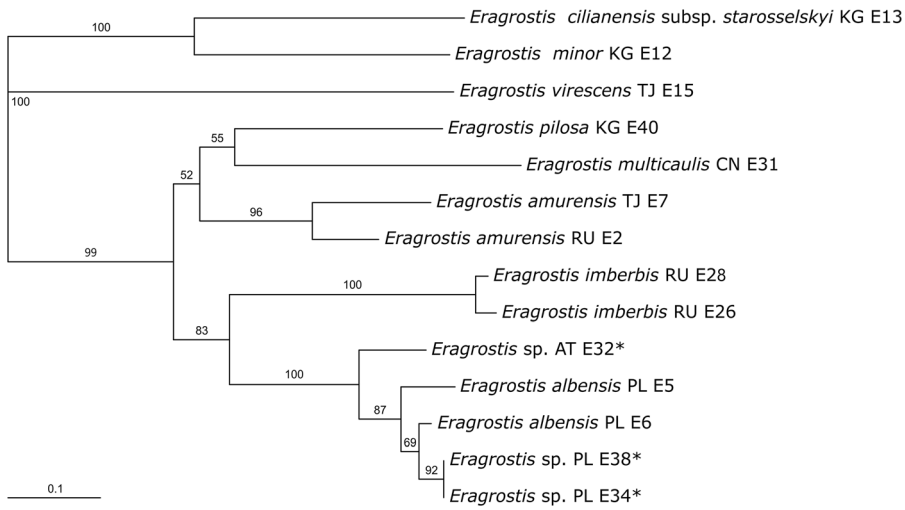

Fig. 3 Neighbour-joining tree of the ISSR profiles of examined *Eragrostis* taxa. The scale bar represents distance between specimens (calculated by means of the Jaccard similarity index). Numbers at nodes represent bootstrap values. Abbreviations: Austria (AT), China (CN), Kyrgyzstan (KG), Poland (PL), Russia (RU), Tajikistan (TJ). [a]Central European glandular morphotype from the *E. pilosa* complex. All examined specimens are listed in "Online Appendix 3"

Table 4 Summary of the ISSR analyses in examined *Eragrostis* taxa

Taxon	Sample	E5	E6	E34	E38	E32	E26	E28	E2	E7	E31	E40	E15	E12	E13	Unique bands
E. albensis	PL E5	48	46	46	46	43	24	25	28	25	18	27	3	3	3	1
E. albensis	PL E6	·	49	48	48	45	25	26	30	25	18	27	3	3	3	0
E. sp. [a]	PL E34	·	·	49	49	44	24	25	29	25	17	26	3	3	3	0
E. sp. [a]	PL E38	·	·	·	49	44	24	25	29	25	17	26	3	3	3	0
E. sp. [a]	AT E32	·	·	·	·	48	25	26	31	26	17	26	3	4	3	2
E. imberbis	RU E26	·	·	·	·	·	27	27	17	15	11	17	1	3	2	0
E. imberbis	RU E28	·	·	·	·	·	·	28	18	15	11	17	1	3	2	0
E. amurensis	RU E2	·	·	·	·	·	·	·	37	32	19	25	3	6	3	0
E. amurensis	TJ E7	·	·	·	·	·	·	·	·	35	18	25	3	6	3	2
E. multicaulis	CN E31	·	·	·	·	·	·	·	·	·	24	20	3	2	1	2
E. pilosa	KG E40	·	·	·	·	·	·	·	·	·	·	39	1	2	1	5
E. virescens	TJ E15	·	·	·	·	·	·	·	·	·	·	·	24	2	2	17
E. minor	KG E12	·	·	·	·	·	·	·	·	·	·	·	·	39	25	9
E. cilianensis	KG E13	·	·	·	·	·	·	·	·	·	·	·	·	·	44	17

Values in diagonal of the table represent the number of bands obtained per specimen; other values represent the number of bands shared by a pair of specimens. The last column shows the number of bands unique to a specimen in an examined set of samples. All of the unique bands within the *E. pilosa* complex were generated using primer M2 (see: Materials and methods). Austria (AT), China (CN), Kyrgyzstan (KG), Poland (PL), Russia (RU), Tajikistan (TJ)

[a]Central European glandular morphotype from the *E. pilosa* complex. All examined specimens are listed in "Online Appendix 3"

Austria, Inn River region, Hagenauer Bucht St. Peter am Hart [Österreich, Oberösterreich, Innviertel, St. Peter am Hart, Hagenauer Bucht, Insels SE des Leitdammdurchstiches], 13°4′36″E/48°16′14″N, MTB:7744/1, Unschärferadius: 100 m, 335 m, 15 September 2013, *M. Hohla* (LI 751048!).

Spread of Eragrostis albensis in Central Europe

Since the first confirmed record of *E. albensis* in Europe (1947, Warsaw, Poland), the species has been observed on over 1250 localities in Central Europe, over 50 in Eastern Europe, and over 10 in Western Europe (Fig. 4). From 1947 to 1979 only three localities were confirmed in Central Europe. In 1980–1999, a considerable spread of *E. albensis* was noted mainly along the Elbe River valley in Germany and along the Vistula River valley in Poland. The species was also found on several localities along the Oder River valley in Germany and on a few other localities in anthropogenic habitats. In the last 20 years, *E. albensis* propagated further especially along the Elbe River and the Oder River (Fig. 5) valleys. It was also observed in many new localities in anthropogenic habitats, particularly along roadsides in Upper Austria, North Tyrol, south and east Poland as well as along rail in south-eastern and east Poland (Fig. 4).

Discussion

The place of origin, distribution and differentiation of the taxa from the *E. pilosa* complex has been already lively discussed but so far with no support of molecular evidence (Špryňar and Kubát 2004; Guzik and Sudnik-Wójcikowska 2005; Pagitz 2012; Seregin 2012a; Hohla 2013). This study indicates that glandular specimens of lovegrasses from Central Europe are more closely related to eglandular *E. albensis* than to glandular *E. amurensis* as was previously suggested (Scholz and Ristow 2005). Therefore, as opposite to the typical eglandular *E. albensis* var. *albensis*, we proposed to delineate a glandular variety of this species under the name *E. albensis* var. *scholziana*. Eglandular *E. albensis* var. *albensis* is much more widespread and occurs widely across Central, Eastern, and part of Western Europe, and is already invasive along several Central European rivers, mainly the Elbe, the Oder and the Vistula. On the other hand, the glandular *E. albensis* var. *scholziana* is so far known only from a dozen of localities in Central Europe and currently is invasive only in the lower section of the Oder River valley.

Results of our work provide another example that illustrates a wide problem concerning a time-delayed identification of spreading organisms which have broad distribution and complicated taxonomy. Such cryptic invaders may remain overlooked or misidentified for a long time, very often until they start to attract more attention after becoming established or problematic in a particular area (Saltonstall 2002; Gerlach et al. 2009; Wong et al. 2018). Another challenge is that lack of solid evidence about history of spread and a place of origin may lead to confusion while classifying a taxon as either native or alien in a specific region (Pyšek et al. 2013; Morais and Reichard 2017).

Unresolved native ranges of E. albensis and E. amurensis

Eragrostis albensis native range has not been conclusively resolved. Scholz (1995) proposed that *E. albensis* could be a Central European young endemic taxon originated as a result of a rapid evolutionary process from a single closely related *Eragrostis* individual introduced by accident from the eastern countries. However, the leading hypothesis suggests that it could rather be an invader, which originated outside Central Europe and invaded this region from the east borders (Špryňar and Kubát 2004).

Like for *Eragrostis albensis*, *E. amurensis* native range has not been resolved yet. Lomonosova (2000) presumed that its westernmost borders reach only Novosibirsk Province, Western Siberia (Russia). On the other hand, Seregin (2012a) proposed to determine native range of *E. amurensis* as reaching further to the west, as far as to SE Belarus and Central and Eastern Ukraine. Our study indicates that previous records of *E. amurensis* from Central Europe are invalid and, therefore, its known distribution does not reach further than to Eastern Europe. However, taking also into account that *E. albensis* is established in European part of Russia, Belarus and Ukraine (Sukhorukov 2011; Seregin 2012a,b), there is a need for a revision of glandular specimens from Eastern Europe and Western Siberia to determine if these specimens indeed represent *E. amurensis* or maybe rather *E. albensis* var. *scholziana*, or even both taxa. Such research could shed more light on actual distribution and native ranges of these two species in Eurasia.

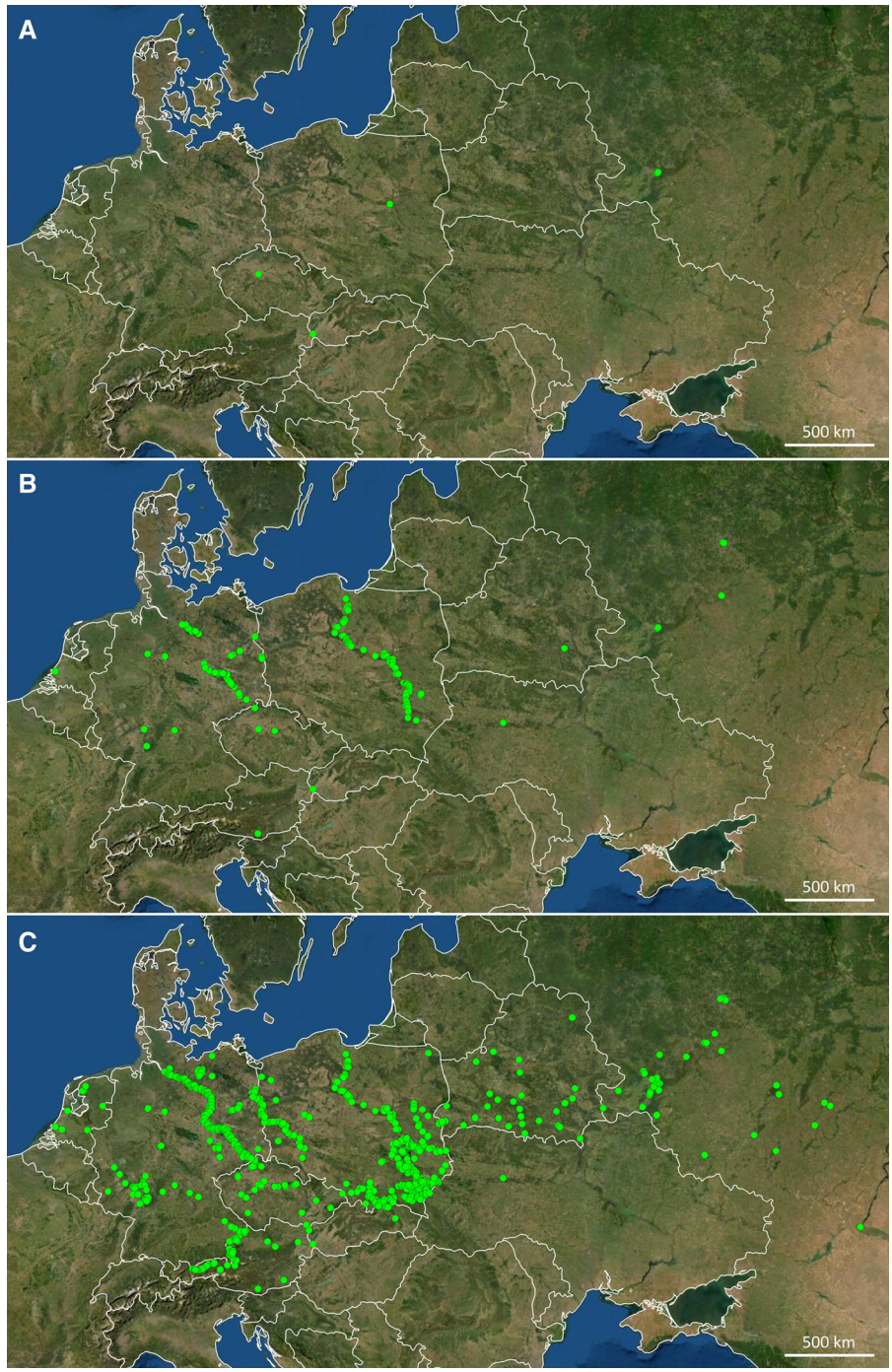

Fig. 4 Spread dynamics of *Eragrostis albensis* in Europe. Localities recorded to 1979 (**a**), 1999 (**b**), and 2020 (**c**). All localities are listed in the "Online Appendix 6"

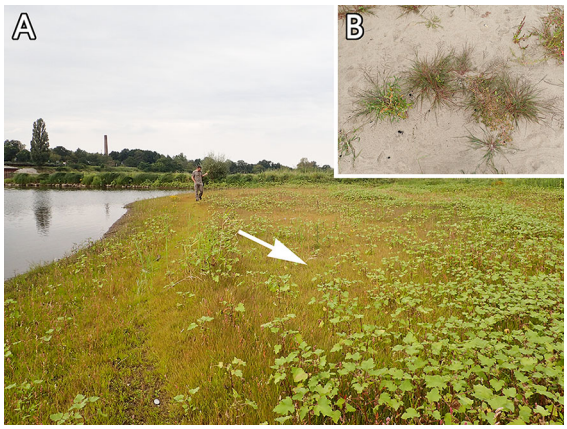

Fig. 5 Riparian communities from the Bidentetea tripartiti class along the Oder River, invaded by *Eragrostis albensis* var. *scholziana* (white arrow) in Słubice, Poland, 52°21′4.28″ N, 14°33′19.32″ E (**a**); mature generative shoots of eglandular *E. albensis* var. *albensis* on sandy banks of the Oder River near Leśna Góra, Poland, 52°01′52.2″ N, 15°38′16.9″ E (**b**); photographs taken 26 August 2017 by A. Wróbel

Eragrostis albensis introduction route to Europe

Probably, the first localities of *E. albensis* in both Eastern and Central Europe were most likely overlooked. Therefore, the data from existing herbarium material could be biased to some extent and should be analysed cautiously. Since the first known registered records in 1947 from Warsaw in Poland, *E. albensis* has become widely established in Central Europe and so far it has been noted in hundreds of localities, most commonly in Germany and Poland. One probable scenario of invasion is that *E. albensis* could have probably invaded Central Europe from the east (Špryňar and Kubát 2004), firstly through anthropogenic habitats and then started to penetrate adjacent riparian communities most likely near roads and bridges (Michalewska and Nobis 2005). Taking into consideration the time of the first confirmed records in Central Europe, an introduction of the species to this region could probably be attributed to military actions during the Second World War in 1940s and accidental dispersal of *E. albensis* seeds from the territory of Russia towards Central European countries. After that, *E. albensis* could have spread further simultaneously through rail, road traffic and along riversides, which was enhanced by dispersal of seeds in sand and gravel gathered along rivers and used in building areas as well as for road maintenance during winter (Michalewska and Nobis 2005). Moreover, floods in XX century might have promoted its rapid propagation along rivers (Guzik and Sudnik-Wójcikowska 2005). In the last 20 years, the species has considerably spread especially in anthropogenic habitats, most likely due to constantly growing road traffic and use of sand from the river banks for road maintenance.

Eragrostis albensis invaded habitats and spreading in Central Europe

Eragrostis albensis is a therophyte which produces numerous small seeds, ca 0.8 × 0.4 mm, which could be dispersed by watercourse, human activities, animals or wind over long distances. Along rivers, *E. albensis* prefers open and sparse alluvial communities including sandy, gravelly and muddy areas on floodplain terraces or river banks exposed during summer low water levels (Dajdok et al. 2018). It occurs abundantly in alluvial habitats and forms dense extensive stands which considerably limit the area for other plants. As a result, *E. albensis* has already outcompeted some of the native species in the region mainly along the Oder (Fig. 4), Elbe, and Vistula River valleys where it has invaded riparian phytocoenoses, including those protected by NATURA 2000—Isoëto-Nanojuncetea, code 3130 and Bidentetea tripartiti, code 3270 (Guzik and Sudnik-Wójcikowska 2005; Krumbiegel 2008; Kącki and Szczęśniak 2009; Dajdok et al. 2018). Moreover, the species could regionally have a negative impact on other therophytes growing on river banks. Spreading *E. albensis* has already been identified as a potential threat to species such as *Corrigiola litoralis* L., *Dichostylis micheliana* (L.) Nees, *Lindernia procumbens* (Krock.) Philcox, and *Lythrum hyssopifolia* L. (Kącki and Szczęśniak 2009; Jackowiak et al. 2014; Dajdok et al. 2018).

According to our research, *E. albensis* var. *scholziana* occurs in much fewer localities compared to *E. albensis* var. *albensis*. Glandular variety occurs both along roadsides and rivers (Scholz and Ristow 2005; Kącki and Szczęśniak 2009; Pagitz 2012; Hohla 2013), and so far, it exhibits invasive potential along the Oder River valley, where it grows abundantly and usually with no admixtures of the typical eglandular morphotype. A spread of *E. albensis* var. *albensis* has been widely observed in Central Europe along rivers,

mainly the Oder, Elbe, and Vistula River valleys as well as in anthropogenic habitats including roadsides, railway tracks, and pavements (Guzik and Sudnik-Wójcikowska 2005; Michalewska and Nobis 2005; Pagitz 2012; Wróbel and Nobis 2017). Taking into account the dynamics of *E. albensis* propagation, it seems very likely that its spread will continue and new localities will be reported soon. Moreover, similarly to other species spreading along motorways and main roads (e.g. *Ambrosia artemisiifolia* L., *Cochlearia danica* L., *Dittrichia graveolens* (L.) Greuter, *Sagina maritima* G.Don, *Senecio inaequidens* DC.; Brandes 2009; Zając and Zając 2019), it is probably a matter of time that *E. albensis* will be observed also in other countries which are adjacent to its currently known distribution.

Glandular morphotypes in the E. pilosa complex

The molecular evidence obtained in this study suggests that the presence of glands as well as hairs, in some cases, may not constitute a species-specific character in *Eragrostis*. Instead, it may rather be regarded as variability within one species. The species-specificity of the morphology, distribution, and abundance of glands as well as hairs has been already the subject of lively debate in botany and is far from resolved (e.g. Van den Borre and Watson 1994; Taia 2006; Ciccarelli et al. 2007; Pagitz 2012; Rola et al. 2019). In the genus *Eragrostis*, the presence of glands (crateriform-like structures, pits, or bands on various parts of a plant) was recognised as a useful and diagnostic morphological character enabling the identification of particular taxa, including in the field (Tutin 1980; Van den Borre and Watson 1994; Peterson 2003; Shouliang and Peterson 2006; Giraldo-Cañas et al. 2012). In the *E. pilosa* complex, the typical *E. pilosa* is considered completely eglandular or at least eglandular on leaf sheaths and blades. It may only sporadically have few glands on a panicle axis or below culm nodes (Koch 1974; Peterson 2003; Giraldo-Cañas et al. 2012; Seregin 2012a). In the complex, presence of glands on leaf sheaths has been related to three taxa so far – *E. perplexa* delimited in the USA as well as *E. amurensis* and *E. voronensis* described from Russia. Densely glandular plants observed in America were proposed as *Eragrostis perplexa* L.H.Harv., (Harvey 1954), subsequently, lowered to the rank of a variety by Koch (1974) as *Eragrostis pilosa* var. *perplexa* (L.H.Harv.) S.D.Koch and regarded by him as restricted to North America. This taxon was distinguished mainly on the basis of presence of abundant glands scattered on a whole plant and characterised by longer glumes, lemmas and caryopsis in comparison to the typical *E. pilosa* (Koch 1974; Peterson 2003). In Eurasia, two species with glandular leaf sheaths were described—*E. amurensis* (Probatova and Sokolovskaya 1981) and *E. voronensis* (Scholz 2010), however, the latter was considered a synonym of the former species after a taxonomic revision (Seregin 2012a). As a consequence, all individuals morphologically assigned to the *E. pilosa* complex in Eurasia but characterised by possession of glands on leaf sheaths have been identified as *E. amurensis* (Scholz and Ristow 2005; Kącki and Szczęśniak 2009; Seregin 2012a).

Nevertheless, our findings support the hypothesis of Pagitz (2012) that *E. albensis* could also express both eglandular and glandular morphotypes which implies that presence of glands on leaf sheaths should no longer be treated as an exclusive character of *E. amurensis* in the *E. pilosa* complex in Eurasia. Moreover, *E. albensis* may be variable across its range and could comprise more than one genetic lineage as the glandular specimens from the Inn River valley in Upper Austria had a slightly different genetic profile and morphology in comparison to plants from the Oder River valley. The next step should be to examine glandular specimens of Pagitz (2012) from North Tyrol, Austria using the ITS marker to determine if they represent the same genetic pattern as Upper Austrian population or form another evolutionary lineage of *E. albensis*.

Although we did not find any hints of recent hybridisation between taxa from the *E. pilosa* complex based on the ITS sequences, more data is needed to test this possibility. In addition, the potential crossing between taxa from the *E. pilosa* complex and more distant *Eragrostis* species should be verified. Probatova and Sokolovskaya (1981) speculated about putative origin of *E. amurensis* and attributed it to hybridisation between eglandular *E. pilosa* and glandular *E. minor*. However, this hypothesis was not supported by molecular evidence by Probatova and Sokolovskaya (1981) and would require further investigation.

Hairy plants in the E. pilosa complex

The characters related to hairs have been widely adopted as diagnostic in the *E. pilosa* complex. A presence of tufts of long hairs on lower leaf sheaths has been indicated as a diagnostic for *E. albensis*, whereas *E. multicaulis* has been generally accepted as not having tufts of long hairs and *E. pilosa* as having them on all leaf sheaths (Scholz 1995; Špryňar and Kubát 2004; Michalewska and Nobis 2005; Nobis and Nobis 2009; Seregin 2012a). During our revision, we found that all specimens of *E. albensis* from Poland, both glandular and eglandular, had tufts of long hairs at the apex only of lower leaf sheaths and did not have long hairs at the uppermost leaf sheaths. In addition, they all had hairs in panicle axils. We also observed that *E. multicaulis* examined from different countries did not have hairs in panicle axils and tufts of long hairs at the apex of leaf sheaths or at most only single long hairs on some leaf sheaths. Such pattern is therefore specific for many populations.

Pagitz (2012) also raised a problem related to the identification of dwarf specimens of *E. albensis* which could be confused with *E. multicaulis* which is regularly smaller and has shorter panicles than *E. albensis*. Such difficulty was also experienced by Scholz who suggested to identify small plants from the Oder River valley as *E. multicaulis* and bigger ones as *E. albensis* (specimens in WRSL herbarium) even though both morphotypes had tufts of long hairs at the apex of lower leaf sheaths. However, Pagitz (2012) noted that *E. albensis* in North Tyrol, Austria partly had tufts of long hairs at the uppermost leaf sheath (which makes these individuals more similar to *E. pilosa* than to *E. albensis*). Similarly, the glandular specimens from the Inn River valley in Upper Austria partly had tufts of long hairs at the uppermost leaf sheaths. That could suggest that there is variability in the expression of hairs in the species from the *E. pilosa* complex which could complicate their identification. As a consequence, collected evidence implies that presence of both hairs and glands could represents regionally variable patterns and, therefore, should rather be treated more cautiously in the taxonomic treatment of the *E. pilosa* complex.

A case of Eragrostis imberbis

The problem concerning classification and distribution of taxa from the *E. pilosa* complex becomes even more complicated if we consider one other taxon, *E. imberbis*. It was described from Russian Far East and was characterised by huge panicles usually longer than the rest of the culm, distinctly long spikelet pedicels, scabrid panicle branches, and eglandular leaf sheaths (Tzvelev 1976; Probatova 1985). Seregin (2012a,b) suggested that Asian *E. imberbis* distributed across Russian Far East and south Siberia might possibly be conspecific with European *E. albensis* and that these two taxa could be one species broadly distributed across Eurasia. *Eragrostis albensis* has never been reported from Asia, however, according to the latest revision, it appeared that it is not possible to indicate clear morphological differences between *E. albensis* and *E. imberbis* (Seregin 2012a,b). *Eragrostis imberbis* can be distinguished from *E. pilosa* mainly by having densely scabrous panicle branches and longer lemma (Probatova 1985; Seregin 2012a), and these two diagnostic differences are similar to those between *E. albensis* and *E. pilosa.* This study confirmed that specimens from Russian Far East, identified as *E. imberbis*, resembled morphology of European *E. albensis* to a large extent. *Eragrostis imberbis* differed only slightly from *E. albensis* in longer pedicels of lateral spikelets and by having usually several verticillate branches at the lowest panicle node (Table 2). Our molecular analyses resolved *E. imberbis* as a distinct although closely related lineage to *E. albensis*. The next step should be to use more sensitive, genome-wide approach and population sampling across Eurasia to get a better insight into the evolutionary history and dispersal dynamics at the population level of species from the *E. pilosa* complex.

Conclusions

Molecular analyses based on ITS and ISSR markers have indicated that the *E. pilosa* complex is genetically variable across Eurasia and there is a possibility to determine species- and lineage-specific genetic apomorphies. The first molecular insight to the complex indicated that *E. albensis* deviates from *E. pilosa* in many traits and, therefore, deserves to be

treated as a separate taxon. The present study also revealed that glandular Central European morphotype, so far classified as *E. amurensis* or *E. albensis*, is genetically more similar to eglandular *E. albensis*, widely established in Central Europe, than to glandular *E. amurensis* described from Asia. Here, we propose to adopt a new taxonomic treatment that *E. albensis* includes both eglandular and glandular individuals, and classify the glandular variety as *E. albensis* var. *scholziana*. Our study implies that presence of glands on leaf sheaths should not be treated as an exclusive character of *E. amurensis* within the *E. pilosa* complex in Eurasia and, therefore, more attention should now be paid to glandular *Eragrostis* plants during identification.

Since the first confirmed records in 1947 till now, *E. albensis* has spread considerably in Central Europe along river valleys, roadsides and railway tracks. Its progressive invasion may potentially pose a threat to native riparian phytocenoses, therefore, regular biomonitoring would be recommended in order to control its impact on local alluvial communities and populations of rare species occurring there.

This study could be a starting point for further large-scale research which is needed to get a full picture of variability within the *E. pilosa* complex across Northern Hemisphere. It would be especially essential to determine whether the glandular specimens recorded from Eastern Europe and Western Siberia do not actually represent the glandular *E. albensis* var. *scholziana*. Further investigation could also address the question if *E. albensis* and *E. imberbis*, resolved as distinct lineages in this study, constitute two closely related but different species.

GenBank accessions

ITS1-5.8 S-ITS2 of nuclear ribosomal DNA.

Eragrostis albensis: MT344699 (E5), MT344698 (E6), MT344694 (E11); *Eragrostis albensis* var. *scholziana*: MT344702 (E3), MT344700 (E4), MT344691 (E14), MT344678 (E32); *Eragrostis amurensis*: MT344701 (E2), MT344697 (E7), MT344680 (E29); *Eragrostis cilianensis* subsp. *starosselskyi*: MT344696 (E9), MT344692 (E13); *Eragrostis imberbis*: MT344682 (E26), MT344681 (E28); *Eragrostis minor*: MT344695 (E10), MT344693 (E12); *Eragrostis multicaulis*: MT344688 (E17), MT344687 (E18), MT344679 (E31); *Eragrostis suaveolens*: MT344686 (E19); *Eragrostis pilosa*: MT344685 (E20), MT344684 (E22), MT344683 (E23); *Eragrostis virescens*: MT344690 (E15), MT344689 (E16).

Chloroplast DNA

petL-psbE intergenic spacer, partial sequence—*Eragrostis albensis* var. *scholziana*: MW019440 (E34); *Eragrostis amurensis*: MW019441 (E2).

rpL32 gene, partial cds; and rpl32-trnL(UAG) intergenic spacer, partial sequence—*Eragrostis albensis*: MW036251 (E6); *Eragrostis albensis* var. *scholziana*: MW036254 (E34); *Eragrostis amurensis*: MW036250 (E2); *Eragrostis multicaulis*: MW036252 (E17); *Eragrostis pilosa* MW036253 (E22).

trnC(GCA)-rpoB intergenic spacer, partial sequence; and (rpoB) gene, partial cds –

Eragrostis albensis: MW036255 (E6); *Eragrostis pilosa* MW036256 (E22).

tRNA-Lys (trnK) gene, intron, partial sequence; and matK gene, partial cds—*Eragrostis albensis*: MW036258 (E6); *Eragrostis amurensis*: MW036257 (E2); *Eragrostis multicaulis*: MW036259 (E17).

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Data availability Data are available in the electronic appendices, and are also available from the corresponding author on reasonable request.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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