Morphometric recognition of *Hordeum murinum* L. subspecies in Slovenia

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Abstract:


Morphometric analysis of Slovenian material belonging to *Hordeum murinum* ssp. *leporinum* and type subspecies confirmed some of already reported distinguishing characters, but majority of them were not measured precisely before. After revision of herbarium material based on results, about 9% of sheets are still somehow »intermediate«, *H. murinum* ssp. *leporinum* is more common in Submediterranean part with some scattered populations in continental Slovenia and the type subspecies occur all over Slovenia in lowland ruderal places. Detailed determination key is provided based on results.

Key words: *Hordeum murinum*, subspecies delimitation, Slovenia

Introduction

Grasses themselves are often neglected even by serious field botanists, particularly so when they are part of ruderal vegetation. This is also the case of *Hordeum murinum* complex, which is probably one of the best studied groups in the genus, however, the taxonomic delimitation of subordinated taxa has always been controversial (Nevski, 1941; Cuda et al., 2013). In Central and SE Europe, *Hordeum murinum* complex represents a taxonomically interesting polyploid group with 4...
taxa recognized at the level of subspecies (or sometimes species). Two of them are rare in the mentioned territory, *Hordeum murinum* ssp. *glauca*um (Stud.) Tzvelev confined to the warmest areas of Southern Europe (e.g. Greece, Cyprus, S Italy, Spain) and a peculiar *H. murinum* ssp. *setariurum* H. Scholz & Raus known only from few localities in N Greece and S Macedonia (Scholz & Raus, 1997; Jogan, 2005). *Hordeum murinum* ssp. *leporinum* (Link) Arcang. and the type subspecies are reported in much wider area with "leporinum" being more thermophilous and particularly common in Mediterranean Europe and the type subspecies widespread but with overlapping range reaching as far South as Peloponnese and N Aegean (Strid, 2016). There are three ploidy levels recognized in the group, diploids (2n=14), tetraploids (2n=28) and hexaploids (2n=42) but the taxonomic delimitation is not congruent with ploidy levels so at least two different ploidy levels are reported for each subspecies, e.g. mostly 2n=28 but also 2n=42 or 2n=14 for both widespread subspecies (Cvelev, 1976; Kankanpaa et al., 1996; Tison & Foucault, 2014; Cuadrado & al., 2013). There is no completely reliable morphological characteristic that distinguishes the recognized subspecies, so several authors refer to the "murinum complex" (Cuadrado & al., 2013). Despite several studies evolution of the polyploid *H. murinum* complex is still not well understood (ibid.) and we can still agree with Nevski (1941), that *H. leporinum* is an ancestor of *H. murinum* s. str., whose spread towards North had been connected to man made ruderalization.

In the territory of Slovenia (and also neighbouring countries) only the the two widespread subspecies are reported, namely *H. murinum* ssp. *murinum* (further Hm) and *H. murinum* ssp. *leporinum* (Hml). In the past, their distribution had been simply recognized as allopatric: Hml reported only for the coastal Submediterranean region of Slovenia and Hm as being excluded from that region and scattered in all other parts (Martinčič, 1984). But some field records blurred that picture with Hml records in the continental Slovenia and also Hm recorded in some localities in the coast (Jogan & al., 1999; Jogan, 2007). Using determination keys it is not always easy to distinguish the two subspecies so our aim was to test the usefulness of morphometric characters for delimitation.

In European floristic works approach in delimitation of the studied two taxa is slightly diverse, either taxa are recognized as independent species (Nevski, 1941; Pignatti, 1983; Martinčič, 1984; Lambinon & al., 1992; Jogan, 2007) or mostly as subspecies (Amaral Franco & Rocha Afonso, 1998; Bolos & Vigo, 2001; Cincović & Kojić, 1976; Ciocirlan, 1990; Conert, 2000; Csiky, 2009; Cuadrado & al., 2013; Fischer & al., 2008; Ilijanić & Topić, 2000; Lauber & Wagner, 1998; Poldini & al., 2002; Stohr, 2002; Tison & Foucault, 2014). Both were mentioned in countries with at least some submediterranean influence, but in Central and W Europe Hml mostly as only casual plant of ruderal sites (Conert, 2000; Lambinon & al., 1992).

Determination keys are mostly using 1-3 characters for delimitation between Hmm and Hml, only some up to 5 characters (Nevski, 1941; Bolos & Vigo, 2001; Jogan, 2007; Lauber & Wagner, 1998). In majority of keys difference in spikelet (and awn) length between central and lateral spikelets of triple is mentioned and quite often also differences in shape of both glumes of lateral spikelet. Often the only mentioned distinguishing measure is length of central spikelet's "stalk". This stalk is a peculiar characteristic in some *Hordeum* species and is developed above glumes and below lemma, so in fact this is a prolonged internode of spikelet axis. Reported gap of this measurement between the subspecies is between 0.6 and 1 mm, inHmm central spikelet stalk is shorter and in Hml longer, mostly with no overlapping reported (Nevski, 1941; Amaral Franco & Rocha Afonso, 1998; Bolos & Vigo, 2001; Ciocirlan, 1990; Fischer & al., 2008; Jogan, 2007; Stohr, 2002; Tison & Foucault, 2014).

In practice it is often not so easy to use this character. Other distinguishing characters used in some of the determination keys were: inflorescence width (Bolos & Vigo, 2001; Lauber & Wagner, 1998) and colour (Lauber & Wagner, 1998; Tison & Foucault, 2014), lateral lemma width (Nevski, 1941; Jogan, 2007) and hairiness (Cvelev, 1976; Jogan, 2014).

**Material and methods**

Herbarium material available in herbarium LJU (University of Ljubljana, Biotechnical Faculty) and personal herbarium collection of the author (HSNJ, Hortus siccus N. Jogan) enriched by some more sheets served as operational taxonomic units (OTUs). In each selected herbarium sheet one well preserved and complete plant served as an OTU and all the measurements taken on that plant. In the measuring phase, one inflorescence was broken apart...
so that all spikelet measurements were done on the triple of spikelets from the central part of inflorescence. Remaining spikes are enclosed in herbarium capsules attached to the sheet. Macroscopic measurements were done by a ruler and all smaller parts measured under stereo microscope Euromex at 20x magnification and with an ocular measuring scale divided to 1/20 mm.

All the distinguishing characters used in above mentioned literature were measured (one measurement per each character per OTU) and in addition to them about as big number of other potentially useful characters as follows:

- VSN: total plant height (dm)
- CPP: palea length of central spikelet (cm)
- CR: length of central lemma's awn (cm)
- CPC: length of central "stalk" (mm)
- CKP: width of central lemma (mm)
- ANT: length of ripe anther (mm)
- LPP: palea length of lateral spikelet (cm)
- LR: length of lateral lemma's awn (cm)
- LPC: length of lateral "stalk" (mm)
- CKP: width of lateral lemma (mm)
- DPP: central palea hairy (Y/N)
- Dstr: margin of outer lateral spikelet glume ciliate (Y/N)
- KOD: length of central spikelet axis prolongation (mm)
- KOS: width of central spikelet axis prolongation (mm)
- KOB: axis prolongation yellow (Y/N)

Palea lengths were taken as equal to lemma lengths, so the lemma's awn lengths were measured from the palea tip to the end of awn. Some peculiar character measurements are shown in Fig. 1.

Input matrix of 86 OTUs with 12 measured characters were used for all subsequent analyses starting with simple univariate statistics and further on different multivariate approaches (using PAST 1.74, http://palaeo-electronica.org/2001_1/past/) to recognize taxonomic structure. A priori formed groups of 2 taxa (Hmm and Hml) were slightly modified after results of numerical analyses so at the end a small "transitional" group of OTUs with unclear taxonomic position had been recognized as the third entity. After removing that group from analyses, the taxonomic structure is much more clear-cut, but in the field we have to be aware of such populations.

Studied material is listed in Supplementum with locality, MTB grid code (Niklfield, 1971), author, date, herbarium acronym and accession number.

**Results and discussion**

Majority of OTUs was determined using the mentioned determination keys but some of them (9%) remain at the level of *H. murinum* s. lat. Those were temporarily excluded from the data matrix for first univariate statistical analyses. From all measured characters, some that showed a potential usefulness for distinguishing studied taxa are represented by box plots in Fig. 2. Usefulness of already reported distinguishing characters has mostly been confirmed for following characters: central stalk length (Fig. 2a), central awn length (Fig. 2b), lateral awn length (Fig. 2c), lateral palea length (Fig. 2d), lateral lemma width (Fig. 2e) and plant height (Fig. 2f). Several of them are reported in determination keys without measurements, only describing their relative length.

Some of the recorded attributive characters were more randomly distributed among studied taxa and results are not presented here because of limited usability for distinguishing taxa.

Several conducted multivariate analyses helped us to recognize the most useful distinguishing characters and also to recognize the overall taxonomic structure. With exclusion of mentioned small group of «intermediates» separation of two clusters representing both subspecies was clear with only slight overlapping as shown in Fig. 3. Obviously delimitation of subspecies will remain uneasy in some populations.

Interesting results turned to be comparison of total lengths of spikelets (Fig. 4), quite often roughly mentioned as »lateral awn exceeding central awn« or »... not exceeding ...«. Total lengths were calculated from 3 measurements for each spikelet (CPC+CPP+CR and LPC+LPP+LR respectively). Indeed in Hmm central awn is mostly slightly

![Fig. 1. Three of the mentioned characters that are specific for Hordeum spikelet triple: CPC (length of central "stalk"), LPC (length of lateral "stalk") and KOD (length of central spikelet axis prolongation)](image-url)
Fig. 2. Box plots of some of the useful distinguishing characters. Box comprising 2nd and 3rd quartile, whiskars 1st and 4th quartile, some outliers marked with *, Hmm on the left (grey), Hml on the right (white). a) central stalk length; b) central awn length; c) lateral awn length; d) lateral palea length; e) lateral lemma width; f) plant height

Fig. 3. Results of PCA. Input data matrix with 12 characters and 86 OTU grouped in Hmm (green squares), Hml (red crosses) and intermediate group (blue triangles)
exceeding the laterals, but in Hml where it is often stated, that lateral awns are exceeding central one, situation is more diverse with only about half of OTU having lateral awns reaching or exceeding the central one. But mostly dimensions of spikelets are distinctly bigger than in Hmm, so probably better distinguishing character for quick orientation in the field would be: total length of spikelets 3-5 cm vs. 5-7 cm in Hmm and Hml respectively. In fact all the average measured values of Hmm and Hml differ mostly 20-35%, an interesting situation that is often observed in polyploids when compared to ancestral diploids, but in discussed group both subspecies are predominantly tetraploid so interpretation must be different.

Our results can be compiled in a determination key which use much more characters than the others mentioned above and some of the measurements are now more precisely measured. But of course we have to bear in mind that Slovenia is a tiny territory where distribution range of both discussed taxa overlap and so populations far from studied area can be slightly different.

Statistics of the useful distinguishing characters are presented as minimum and maximum (in brackets) and an interval between 1st and 3rd quartile.

<table>
<thead>
<tr>
<th>Character</th>
<th>Hmm</th>
<th>Hml</th>
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<tbody>
<tr>
<td>Total spikelet lengths</td>
<td>3.5-4.6 (5.9) cm</td>
<td>central spikelet of a triple with</td>
</tr>
<tr>
<td>lemma</td>
<td>1 (1) 1 cm long, lateral spikelets with lemma's awn (1.4) 2-3 (3.5) cm long, palea (0.75) 0.9-1.1 (1.2) cm long and lemma (1) 1.2-1.6 (1.8) mm wide. Anthers (0.6) 0.7-1 (1.4) mm, plant (20) 25-45 (60) cm</td>
<td></td>
</tr>
<tr>
<td>palea</td>
<td>8 (5) 0.7 cm long, lateral spikelets with lemma's awn (1.4) 2-3 (3.5) cm long, palea (0.75) 0.9-1.1 (1.2) cm long and lemma (1) 1.2-1.6 (1.8) mm wide. Anthers (0.6) 0.7-1 (1.4) mm, plant (20) 25-45 (60) cm</td>
<td></td>
</tr>
<tr>
<td>lemma's awn</td>
<td>2.4-3.5 (4) cm long, lateral spikelets with lemma's awn (1.4) 2-3 (3.5) cm long, palea (0.75) 0.9-1.1 (1.2) cm long and lemma (1) 1.2-1.6 (1.8) mm wide. Anthers (0.6) 0.7-1 (1.4) mm, plant (20) 25-45 (60) cm</td>
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Horizontal spikelet (cm)

Vertical spikelet (cm)

Hmm

Hml

**Fig. 4.** Comparison of total spikelet lengths of central vs. lateral spikelet of triple. Hmm represented with grey, Hml with empty symbols. Equal lengths at dashed diagonale, longer central spikelet below

**Fig. 5.** Distribution pattern matches quite well pattern in adjacent Friuli-Venezia Giulia (NE Italy, Venezia Giulia (NE Italy, al., 2013) for which no herbarium material had been available so it is better to recognize them on that taxonomic level. Obviously the group is represented only in lowland Slovenia mostly below 300 m a.s.l., only few scattered localities can reach up to 600 m a.s.l. along the main highways. In the extreme SW part along the Adriatic coast and in Vipava valley Hml is the common taxon, but also some populations of Hmm can be found. In central and E Slovenia Hmm is present in majority of sampling sites, but there are also some records of Hml populations.

As it has been expected that both studied taxa are mostly tetraploids, possible hybridization could not be excluded as a cause for "intermediate" populations, but detailed study of those "intermediate" OTUs was beyond our scope. It is important to stress that in the discussed territory both studied taxa are sympatric with slightly bigger frequency of Hml populations in the SW coastal part of Slovenia and Hmm present in all lowland parts of the country. In interpreting "intermediate" also a hypothesis of Ne v s k i (1941), that Hml is an ancestral relative of Hmm can be roughly taken into consideration, but it seems that the evolution of the »murinum complex« had been more complicated (Cu a d r a d o & al., 2013)
In continental adjacent countries, Austria and Hungary, Hml occurrence is very local to ephemeral (Csiky, 2009; Fischer et al., 2008). In the future presence of Hml in continental Slovenia is to be studied in detail as it would not be possible to exclude the probability of mostly ephemeral occurrence of this taxon in extremely dry ruderal places as e.g. along the railways or in abandoned gravel sites in bigger cities where "urban heat island" phenomenon can impact local mesoclimatic conditions. On the other hand it would be interesting to study populations of Hmm in the coastal part of Slovenia. At least the impression after the sampling is that Hmm is more linked to shadowy, slightly wet ruderal places whereas Hml is common in very dry ruderal communities.

Conclusion

As a result we can confirm, that both previously mentioned subspecies of *H. murinum* are present in Slovenia, there are more useful distinguishing characters than reported before but still it is not always easy to recognize the subspecies. *Hordeum murinum* ssp. *murinum* is present all-over Slovenian lowland and *H. murinum* ssp. *leporinum* predominantly in the coastal region, but with some scattered populations also inland. Ecological preferences in the areas where both co-occur remained to be studied.

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