CONTRIBUTION TO THE KNOWLEDGE OF BROMUS DIANDRUS–RIGIDUS (POACEAE) POLYPLOID COMPLEX IN THE BULGARIAN AND ROMANIAN FLORAS

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Abstract

Comparative morphological and ploidy level survey of the polyploid complex B. diandrus-rigidus in the Bulgarian and Romanian floras was performed. Critical examination of the morphology of the collected specimens revealed B. diandrus is represented in both floras. The species has already been reported for Romania under the name B. rigidus. For Bulgaria, the species has been very recently reported on a conference poster with a single gathering from 2020. Here, we provide more records of the species along the whole Bulgarian Black Sea coast floristic region, with the oldest gathering dating back to 2003. Ploidy level estimation of the Bulgarian accessions by flow cytometry revealed all studied populations are octoploid which is congruent with data from elsewhere, and confirms B. diandrus is present in the Bulgarian flora. For both the Bulgarian and Romanian floras alien status of the species has been inferred.

Key words: Anisantha, Black Sea coast, Bromus diandrus-rigidus complex, Bulgaria, genome size, new records, Romania

Introduction. The annual species of genus Bromus L. with cuneate spikelets and lemma awns at least twice longer than the caryopsis are traditionally placed in Bromus sect. Genea Dumort. The latter is treated within a distinct genus Anisantha K. Koch in some recent sources (e.g. [1]). In Bulgaria, this section is
represented by *B. sterilis* L., *B. tectorum* L. and *B. madritensis* L. [2], and in Romania – by *B. sterilis*, *B. tectorum* and *B. rigidus* Roth [3]. All these species are part of a taxonomically challenging polyploid complex with reticulate origin of the polyploid taxa [4].

In March 2019, during a meeting at the herbarium in Cluj-Napoca (Romania), Gavril Negrean drew the attention of the first author with his 2008 finding of “*Bromus rigidus*” from the Bulgarian Black Sea coast near the town of Balchik. He provided his material with a request to further study and jointly report this new for the Bulgarian flora species record. During field research in 2019–2021 eight localities of the species (including the one found by Negrean) in Bulgaria were recorded. However, doubts have arisen whether the species indeed belongs to *B. rigidus* s.str. Another species – *B. diandrus* Roth is morphologically very similar and belongs to the same intricate polyploid complex. Both *B. rigidus* and *B. diandrus* differ from the other members of the *B. sect. Genea* by the length of their lemma being at least 20 mm, usually in the range 22–28 mm. According to the key in Flora Europaea [5], they can be distinguished from each other by the habit of the panicle, which is dense, stiffly erect in *B. rigidus*, and usually lax, spreading, somewhat loose in *B. diandrus*. In addition, NADERI et al. [6] indicate as a key diagnostic feature the length of the branches of the panicle – branches mainly shorter than spikelets in *B. rigidus* and branches mainly longer than spikelets in *B. diandrus*.

The variations in the above diagnostic morphological features observed in the field did not provide convincing evidence to which of the two species the studied Bulgarian populations belong to and raised the question of whether both species – *B. rigidus* and *B. diandrus* – are actually represented. Also, it is worth mentioning that the widespread species *B. sterilis* was found to co-occur in almost all studied localities.

The aim of the article is to revise the *B. diandrus*–*rigidus* polyploid complex in the Bulgarian and Romanian floras, based on morphological and ploidy level studies, and to clarify which taxa are actually present, as well as to report their currently known distribution ranges.

**Materials and methods.** Plant material has been collected from various localities in the Black Sea coast floristic region of Bulgaria and from two sites in Romania. Data about the morphology of the species has been obtained from the studied personal gatherings and from the herbarium specimens in the herbaria CL, SO, SOA and SOM (acronyms according to [7]). The collected specimens have been deposited in the herbarium SOM.

Ploidy level was estimated by flow cytometry using CyFlow SL Green (Partec, Germany) equipped with a green (532 nm) solid-state laser. Usually, several live specimens, collected and kept in wet tissue paper in a refrigerator for a few days until required, have been measured per population. Plant material was treated with CyStain PI Absolute P extraction and staining kit (Sysmex), following the
protocol provided in the kit. The main steps were described in [8]. *Pisum sativum* ‘Kleine Rheinländerin’ (1C = 1Cx = 4.38 pg, [9]) was used as internal standard. The samples were then measured at a rate of 10–20 nuclei per second, with 5000 nuclei for each measurement; three replicates were done for measurements with CV to 3%, and five replicates for those with CV = 3–5%. The terminology for the C-value follows Greilhuber et al. [10].

Although we are aware of the modern treatment of the studied taxa under the genus *Anisantha* by some authors (e.g. [1]), we preferred to retain here the traditional taxonomic treatment under *Bromus* for compatibility with all the existing Bulgarian and Romanian Floras and field guides.

Results and discussion. Morphology. Within the group of *B. sterilis* – *B. diandrus* – *B. rigidus*, *B. sterilis* is the easiest to recognize. It is more or less stable morphologically and is characterized by a very lax panicle, branches mostly nodding and much longer than spikelets. The closely related *B. rigidus* and *B. diandrus* are quite similar to each other and variable in their morphology. The taxonomic delimitation of the last two species, based on morphological characters, is weakly resolved and still obscure.

Studying the morphology of the panicle, it was found that the majority of the Bulgarian populations have loose panicles with erecto-patent branches, slightly shorter or equal to the length of spikelets. These traits are attributed to typical *B. diandrus* (Fig. 1A). Deviation was observed only in the population at Kranevo village, in which some individuals had erect panicle with branches subsessile or 3–4 times shorter than spikelets (SOM 177307), which in turn is associated with *B. rigidus*. However, since these plants had the same ploidy level as the other individuals in the population which had branches as long as spikelets, they all should be regarded as *B. diandrus*.

Another morphological character to distinguish the pair *B. diandrus* – *B. rigidus* is the scar shape of the rachilla segment in the floret. Smith [5] stated that callus-scar in *B. diandrus* is almost circular, while in *B. rigidus* it is more or less elliptical. Böcker et al. [11] also reported that ovate shape is characteristic for *B. diandrus*, whereas in *B. rigidus* the scar shape is elongated. They argued the callus-scar is a reliable diagnostic character. In contrast to the previous authors, Sales [12] considered the callus-scar to be quite variable and an uncertain character for distinguishing of the two species. Our investigation confirmed Sales’s [12] observations and also found that the shape is rather variable, even within one population, from narrowly elliptical to ovate with a pointed tip.

*Bromus rigidus* has already been reported from Romania [3,5,20]. Judging from the morphology of the examined herbarium material, it can be concluded that the specimens resemble more closely *B. diandrus* and most likely belong to the latter species.

Genome size and ploidy level determination. Diploid chromosome number with 2n=2x=14 was published for *B. sterilis* from the Black Sea coast.
Fig. 1. *Bromus diandrus*: A. panicle; B. dense stand of the species in a ruderal place near a sandy beach
Table 1

<table>
<thead>
<tr>
<th>No</th>
<th>Species/Locality*</th>
<th>Number of plants measured</th>
<th>1C [pg]</th>
<th>1Cx [pg]</th>
<th>Ploidy level</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Bromus diandrus</em></td>
<td>Balchik town, 22.05.2020, S. Stoyanov</td>
<td>5</td>
<td>11.80</td>
<td>2.95</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>Dolphin camping area, 21.05.2020, S. Stoyanov</td>
<td>7</td>
<td>12.13</td>
<td>3.03</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>Arkutino Resort, 21.05.2020, S. Stoyanov</td>
<td>7</td>
<td>11.70</td>
<td>2.93</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>Gradina camping area, 21.05.2020, S. Stoyanov</td>
<td>6</td>
<td>12.13</td>
<td>3.03</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>Kabakum beach, 02.05.2021, V. Vladimirov</td>
<td>1</td>
<td>11.52</td>
<td>2.88</td>
</tr>
<tr>
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<td>Balchik town, 22.05.2020, S. Stoyanov</td>
<td>6</td>
<td>3.13</td>
<td>3.13</td>
</tr>
<tr>
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<td></td>
<td>Dolphin camping area, 21.05.2020, S. Stoyanov</td>
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<td>3.07</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>Arkutino Resort, 21.05.2020, S. Stoyanov</td>
<td>6</td>
<td>3.13</td>
<td>3.13</td>
</tr>
</tbody>
</table>

*For full citation of the localities, see ‘Studied specimens’

(Northern) floristic region [13,14]. The established genome size varies from 1C = 3.07 pg to 1C = 3.13 (see Table 1), which corresponds to an earlier report of 1C = 2.98 pg [15]. The measured genome size in the Bulgarian accessions of *B. diandrus* is in the range 1C = 11.52–12.13 pg (Table 1) which is congruent with earlier data, e.g. 1C = 11.90 pg [16], and corresponds to octoploid level. At least two ploidy levels have been reported in each of the three species of consideration: *B. sterilis* – 2n = 2x (most common), 4x; *B. rigidus* – 2n = 4x, 6x, and *B. diandrus* – 2n = 6x, 8x [4,17]. The morphological distinction of the different cytotypes is often rather difficult [17], hence, the delimitation of the polyploid species is not always clear. Therefore, some authors suggest infraspecific treatment of “*B. diandrus*” and “*B. rigidus*” under *B. diandrus* which is the priority name at species level ([17] and references therein). So far, the octoploid level has been commonly associated with *B. diandrus*. Both the morphology of the studied material and the detected octoploid level suggest that the Bulgarian plants belong to *B. diandrus* s.str. and are reported here under this name. Unfortunately, we did not have the opportunity to study the ploidy level in any Romanian accession of the species.

**Distribution and probable origin of the populations of *B. diandrus* in Bulgaria and Romania.** It was found that in Bulgaria *B. diandrus* is distributed almost along the entire Black Sea coast floristic region, from the town of Balchik in the north to the town of Ahtopol in the south. During the field work, eight populations of the species were registered (see ‘Studied specimens’). While preparing this manuscript, the taxon was reported on a conference poster from...
another locality, Irakli beach, recorded in the summer of 2020 \cite{18}. The species is mainly confined to sandy substrates and, in addition to being a typical psammophyte, it is also found in the composition of ruderal grass communities adjacent to the dune habitats (Fig. 1B).

The present wide distribution of \textit{B. diandrus} raised the question about its origin in the Bulgarian flora – native or alien. The botanical interest in the coastal territories dates back to the beginning of the 20th century. If we assume that the species was spread on the Bulgarian dunes even at that time, it would have been collected by the early Bulgarian botanists and possibly misidentified for the morphologically similar \textit{B. sterilis}. Careful examination of all the \textit{Bromus} material deposited in the three Bulgarian herbaria SO, SOA and SOM did not reveal any sample from the \textit{B. diandrus–rigidus} complex. A specimen of \textit{B. diandrus} collected from the dunes of Arkutino resort was erroneously identified as \textit{Vulpia fasciculata} (SOM 158470). This turned out to be the first herbarium record of \textit{B. diandrus} from Bulgaria and dates back to 2003. Such a late recording of this species in Bulgaria speaks more in favour of its alien origin in the Bulgarian flora. It can be inferred that \textit{B. diandrus} was introduced to the country at least two decades ago. PETROVA et al. \cite{19}, who reported \textit{Vulpia fasciculata} for the first time in the Bulgarian flora, also inferred an adventitious origin of this psammophytic species, possibly due to the increasing use of the coastline and beach strips for recreation. They registered localities mainly around the large resorts, Sunny Beach, Sozopol, Primorsko, etc., and concluded that this species most likely was accidentally introduced by humans.

Alien status is suggested for the Romanian occurrences of the species as well. In fact, in 2008 the second author collected material from the \textit{B. diandrus–rigidus} complex from both the Bulgarian and Romanian Black Sea coasts. The locality from the Constanța region (Romania) he considered adventitious and later published the species under the name “\textit{B. rigidus}” as part of the list of alien species from the port of Constanța \cite{20}. Until then, “\textit{B. rigidus}” was known only from the southwestern part of Romania, Caraș-Severin, reported in the early 20th century by Janka \cite{3}, noting that the species originated from the Western Mediterranean, i.e. even then the species was considered an alien. Current data confirms presence of \textit{B. diandrus} in only a few localities in Romania.

\textbf{Studied specimens}

\textit{Bromus diandrus}

\textbf{Bulgaria. Black Sea coast (Northern):} Dobrogea, Balchik, ad littore Mare Nigrum, sub Castellum Regiae Maria, in arenosis, 30.04.2008, \textit{G. Negrean} (SOM 177293, sub \textit{B. rigidus}); Dobrich district, Balchik town, at the entrance of central beach of Balchik, on ruderal sandy places, 43.40367°N, 28.16901°E, 22.05.2020, \textit{S. Stoyanov} (SOM 177302, 177303); Varna city, Chayka Resort, on and by the beach south of hotel Zhurnalist, 43.25976°N, 28.03291°E, 27.05.2020, \textit{V. Vladimirov} (SOM 177304, 177305); Dobrich district, on the beach between Al-
bena Resort and Kranevo village, 43.34805°N, 28.07250°E, 29.05.2020, V. Vladimirov (SOM 177306, 177307); Varna city, Kabakum beach at Chayka Resort, on the beach, 43.256625°N, 28.030911°E, 02.05.2021, V. Vladimirov obs.; **Black Sea coast (Southern):** Burgas district, at Arkutino locality, on dunes, 21.05.2003, A. Petrova (SOM 158470, sub Vulpia fasciculata); Burgas district, Ahtopol town, at the entrance of central beach of Ahtopol, on coastal sand dunes, 42.10164°N, 27.93379°E, 21.05.2020, S. Stoyanov (SOM 177294, 177295); Burgas district, northwest of Ahtopol town, in the area of camping Dolphin, on coastal sand dunes, 42.10278°N, 27.92400°E, 21.05.2020, S. Stoyanov (SOM 177296, 177297); Burgas district, Arkutino Resort, along the main asphalt alley, on ruderal sandy places, 42.33381°N, 27.72778°E, 21.05.2020, S. Stoyanov (SOM 177298, 177299); Burgas district, south of Chernomorets village, in the area of camping Gradina, on ruderal sandy places, 42.42719°N, 27.64454°E, 21.05.2020, S. Stoyanov (SOM 177300, 177301).

**Romania.** District Constanța, Portul [harbour] Constanța, in locis ruderalis, 12.05.2008, G. Negrean (SOM 177292, sub B. rigidus); District Mehedinți, Drobeta-Turnu Severin, Gara [railway station], in locis ruderalis, 44°37′18.58″N, 22°38′03.39″E, 52 m, 25.06.2013, G. Negrean (CL 665457, photo!, sub B. rigidus).

**Bromus sterilis**

**Bulgaria. Black Sea coast (Northern):** Dobrich district, Balchik town, at the entrance of central beach of Balchik, on ruderal sandy places, 43.40367°N, 28.16901°E, 22.05.2020, S. Stoyanov (SOM 177311); Varna city, Chayka Resort, on and by the beach south of hotel Zhurnalist, 43.25976°N, 28.03291°E, 27.05.2020, V. Vladimirov (SOM 177312); Varna district, on the beach north of the mouth of River Kamchiya, 43.02428°N, 27.88883°E, 30.05.2020, V. Vladimirov (SOM 177313); **Black Sea coast (Southern):** Burgas district, northwest of Ahtopol town, in the area of camping Dolphin, on coastal sand dunes, 42.10278°N, 27.92400°E, 21.05.2020, S. Stoyanov (SOM 177308); Burgas district, Arkutino Resort, along the main asphalt alley, on ruderal sandy places, 42.33381°N, 27.72778°E, 21.05.2020, S. Stoyanov (SOM 177309, 177310).

**REFERENCES**


