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Bryology in Serbia: from floristics to chemosystematics

Review Article

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Abstract:

Bryological research in Serbia can be divided into several periods and research fields, the first of which being floristic research. An important exploration of bryophytes in Serbia started at the end of the 19th century, and with a few discontinuances it continued up today. During the last decade of the 20th century couple of researchers started their work on the exploration of bryophytes. They focused on bryologically important habitats like sources, riverbanks, peat bogs and mountains. A significant number of new species was reported for the country, but the revision of the old records has been done as well, hence today flora of Serbia numbers 831 bryophyte taxa in total. Progress of the botanical research has brought interdisciplinarity into bryology. As a result, bryology in Serbia nowadays spread in different types of bryophyte research. For example, bryophytes are studied to investigate the biological, antimicrobial, antifungal and cytotoxic activity, biotransformation of molecules, phytochemical composition and as model organisms in stress physiology. Special emphasis is also given on active conservation of the species. This represents only some directions of what is currently studying on bryophytes in Serbia.

Key words:

bryophytes, Serbia, history, floristics, phytochemistry, biological activity

Apstract:

Kolekcija mahovina BEOU - zanemareno nacionalno istraživačko blago

Istoriju briologije u Srbiji možemo podeliti na nekoliko perioda i pravaca. Prvi je pravac florističke (inventarizacione) briologije. Naime značajnija briološka istraživanja u Srbiji započeta su krajem devetnaestog veka i sa znatnim prekidima traju do danas. U poslednjoj dekadi dvadesetog veka pojavilo se nekoliko istraživača koji su se prevashodno posvetili istraživanju mahovina. Istraživanja su usmerena na briološki zanimljiva staništa kao što su vrela, reke, tresetišta, planine. Otkriven je značajan broj novih taksona ali izvršena je i revizija nekih ranijih podataka, tako da trenutno brioflora Srbije broji 831 takson. Kako je opšti način botaničkih istraživanja napredovao, i u briologiji se prešlo na multidisciplinarnost. Grupa mlađih istraživača bavi se istraživanjima biološke aktivnosti mahovina, antimikrobne, antifungalne i citotoksične, fitohemijom mahovina, biotransformacijom molekula izolovanih iz mahovina ali i fiziologijom stresa. Poseban akcenat je stavljen na aktivnu zaštitu i reintrodukciju. Ovo je samo deo onoga što se danas iz Briologije radi u Srbiji.

Ključne reči:

briofite, Srbija, istorija, floristika, fitohemija, biološka aktivnost

Introduction

Bryological research in Serbia could be divided into three periods of investigation, and several research fields. First period coincide to the onset of the botany in Serbia, or with the work of Josif Pančić and his students. This period is characterized mainly by floristic or inventory studies, with relatively little bryophyte records made. After a brief break in investigation, research slightly intensified during the period from 1920s to 1960s with the work of group of phytocenologists. Third, or the modern period, began in the end of 20th century, and is the most fruitful period so far (Pantović & Sabovljević, 2017).

During this period, in addition to extensive floristic research, ecological investigations, the biological activity of moss extracts, their phytochemical analysis, as well as the transformation of molecules isolated from the extracts, were also initiated and successfully realized. At present, a lot of research is conducting in the field of stress physiology in Serbia, as well. A group of researchers is intensively working on the conservation of bryophytes, with an emphasis on active protection and reintroduction of the rare and threatened species. The Institute of Botany and Botanical Gardens "Jevremovac" has valuable collection of over 260 species of living bryophytes from around the world, of which about 60% are regionally or globally endangered.

Floristic-ecological research of the bryophytes of Serbia

As a part of general botanical research in the territory of Serbia, research of the bryophyte flora had started in the middle of the 19th century. The first bryophyte records were published in the works of the researchers of that time (Grisebach, 1843; Pančić, 1859, 1863). More extensive research of bryophyte flora has began at the end of the century when Svetozar Obradović, Đura Ilić and Lujo Adamović started researching bryophytes; they were sending most of the collected material for determination and verification by more experienced botanists (Wettstein, 1890; Simić, 1892; Matouschek, 1899, 1901). Simić (1897, 1898) published part of his research on the Vranje surrounding in the reports of the Vranje Gymnasium. Schiffner (1897) identified one species from the Bornmuller's herbarium. Research results obtained during this period were summarized by Jurišić (1900) and Simić (1900). In these papers, they included part of their unpublished results as well - Jurišić cites 209 and Simić 145 species. For the next ten years, bryological research was done mostly by Katić (1900, 1903, 1906, 1907a, 1907b i 1909) and Košanin (1909a, 1909b, 1910).

After this period, bryological studies were done by Podpera (1922), Černjavski (1929, 1932, 1937, 1938), Pichler (1931, 1939, 1940), Grebenschikov (1943, 1949, 1950), Soška (1949), Knapp (1944), Rudski (1949a, 1949b) and Rajevski (1951). Pavletić (1955) stated in his work "Prodromus of the bryophyte flora of Yugoslavia" 374 taxa (57 Marchantiophyta, 317 Bryophyta) to be present in Serbia.

Since the 1950s, only sporadic bryophye records were published within the general floristic research (Gigov & Nikolić, 1954; Gigov, 1956; Slavnić, 1956; Pavletić, 1956, 1968; Janković & Janković 1962; Blečić & Tatić, 1962; Čolić et al., 1963; Pavlović, 1951, 1964; Mišić & Popović, 1960; Popović, 1966; Filipović, 1966; Guelmino, 1970, 1972; Erdeši, 1971; Babić, 1972; Tešić et al., 1979; Martinčič, 1980; Janković & Mišić 1980; Jovanović 1980; Janković & Stevanović, 1981; Mišić, 1982; Grgić, 1983; Gajić, 1983, 1986, 1988, 1989; Gajić & Karadžić, 1991; Petković et al., 1988, 1990, 1991; Mišić & Panić, 1989). Based on these studies, the list of bryophyte flora has been updated by Gajić et al. (1991), who cited 415 bryophyte species in Serbia (60 Marchantiophyta, 355 Bryophyta).

The third period (or modern period) of bryophyte research began in the last decade of the 20th century when several researchers devoted their research to the bryophytes: Ranđelović, 1994; Stevanović et al., 1995; Veljić et al,. 1996, 2001a, 2001b, 2004, 2006, 2008, 2013, 2016a, 2016b; Veljić & Marin, 1997; Pavić et al., 1998; Sabovljević & Stevanović, 1999; Petković et al., 2000; Sabovljević, 1998, 1999, 2000, 2003a, 2003b, 2006; Papp & Sabovljević, 2001, 2002, 2010; Grdović & Blaženčić, 2001; Sabovljević & Cvetić, 2003; Pócs et al., 2004; Cvetić & Sabovljević, 2005, Papp & Erzberger, 2005; Grdović, 2005; Grdović & Stavretović, 2004, 2006; Sabovljević & Grdović, 2009; Papp & Erzberger, 2005, 2007a, 2007b, 2009; Erzberger & Papp, 2007; Papp et al., 2004, 2006, 2012a, 2012b, 2013, 2014a, 2014b, 2014c, 2016a, 2016b, 2016c; Ranđelović, Zlatković, 2010; Pantović & Sabovljević, 2013, 2017; Pantović et al., 2014; Ilić et al., 2015. Research was focused on biologically interesting and unexplored habitats such as springs, rivers, peatlands, mountains. This phase of intensive research of the flora and ecology of bryophytes is ongoing, and more recently two Ph.D. dissertations focusing on bryophytes in Serbia were done: "Biogeographic and ecological study of the bryophyte flora of Serbia" (Pantović, 2018) and "Diversity, distribution, differentiation of micro-habitats and the structure of moss communities of Fruška gora mountain" (Ilić, 2019). In this short period of time,

Fig. 1. Collection of bryophytes in the BEOU Herbarium. Shown are digitized specimens included in the Bryo database (photo J. Pantović)

Fig. 2. Reintroduction of *Henediella heimii* (marked red) and *Enthostodon hungaricus* (marked yellow), propagated axenically *in vitro*, acclimatized and finally released to nature on potentially suitable site in Banat, Serbia! (photo M. Sabovljević)

a significant number of new taxa has been reported for the country, but also some previous data has been revised, so Serbia currently numbers 830 bryophyte taxa (1 Anthocerotophyta, 142 Marchantiophyta, 687 Bryophyta).

Historical data on the distribution and ecology of bryophytes has been collected and digitized into a unique database of bryophytes of Serbia, the Bryo database (**Fig. 1**). Updated lists of liverworts and mosses of Serbia have been prepared and are currently in press.

Protection and conservation of bryophytes

Bryophytes, like all other living organisms, are exposed to various negative environmental factors. They are threatened similarily as vascular plants, but the attention and concern for their conservation is much lower. Among bryophytes, there are taxa that are extinct, in different categories of threat, or rare, which has been confirmed by the increasing number of the regional Red Lists (Sabovljević et al., 2004; Marka et al., 2012; Hodgetts et al., 2019). Recognizing this global problem, a group of associates gathered around Marko and Aneta Sabovljević (BBGB - Bryophyte Biology Group Belgrade) has developed into the widely recognized group for bryophyte conservation (Sabovljević et al., 2009; Sabovljević et al., 2014; Vujičić et al., 2012). The Institute of Botany and Botanical Garden "Jevremovac" has an *in vitro* living collection of over 260 species of bryophytes from around the world, of which about 60% are regionally or globally endangered. Nearly 52% of the species in the collection are on the European and regional red lists.

Special attention is given to the active protection, like reintroduction and population strengthening of the rare and endangered taxa. *In vitro* propagated and grown plants are being prepared for reintroduction to the potentially suitable habitats. Several species such as *Hendiella heimii* and *Entostodon hungaricus* (**Fig. 2**) have been propagated this way and reintroduced to habitats in Banat, Serbia (Sabovljević et al., 2012a, 2012b, 2016, 2018).

Ecophysiological research of bryophytes in Serbia

With progress of the botanical research, a multidisciplinary approach has been adopted in bryology as well. A group of young researchers has started working on bryophyte stress physiology (Rajčić et al., 2015; Vujičić, 2016; Vujičić et al., 2016; Ćosić et al., 2018). As a part of these studies, a doctoral dissertation entitled "Biochemical and ecophysiological properties of selected moss species during salt stress in the axenical conditions" (Vujičić, 2016) has been done. Monitoring the parameters of morphogenesis revealed that bryophyes and vascular plants respond differently to the short-term and long-term salt stress, indicating different survival strategies with the increased salinity of the microenvironment. The effect of salt stress is also observed by the change in the concentration of tocopherols in mosses (*Physcomitrella patens, Entosthodon hungaricus* and *Henediella heimii*) (**Fig. 3**). Namely, tocopherol as a non-enzymatic component plays an important role in the moss response to the stress caused by increased NaCl concentration. The results of the study show that under long-term stress, the concentration of tocopherols increases on moderately saline media (50-100 mM NaCl) in *H. heimii*. The salt stress affects concentration of proline and the ratio of proline and free amino acids in mosses (*P. patens, E. hungaricus* and *H. heimii*). Proline as an essential amino acid plays a very important role in protecting the cell from osmotic stress, and it is involved in osmotic adaptation. It was discovered that proline concentration increases with the increase in salt concentration in all tested species during short-term stress, and that the ratio of proline and free amino acids increases with the increase in salt concentration in the medium. Long-term salt stress induces a decrease of proline concentration in plants grown on a medium with the high concentration of

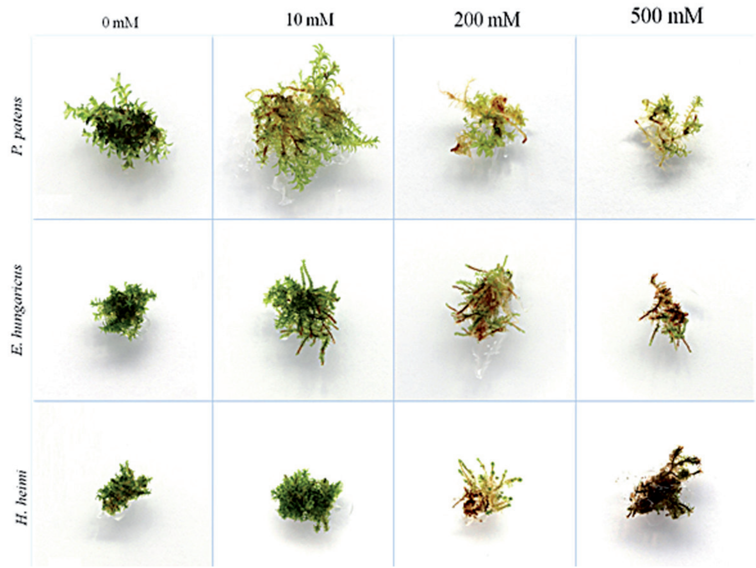

Fig. 3. The appearance of the investigated species cultivated for three weeks in culture in vitro on a nutrient medium with the addition of various salt concentrations (photo M. Vujičić)

salt. During long-term stress, the ratio of proline to free amino acids also increases with the increase of salt concentration in the medium. In addition to the non-enzymatic components, as a response to the salt stress enzyme activity (peroxidase, catalase, and superoxide dismutase) was monitored as well.

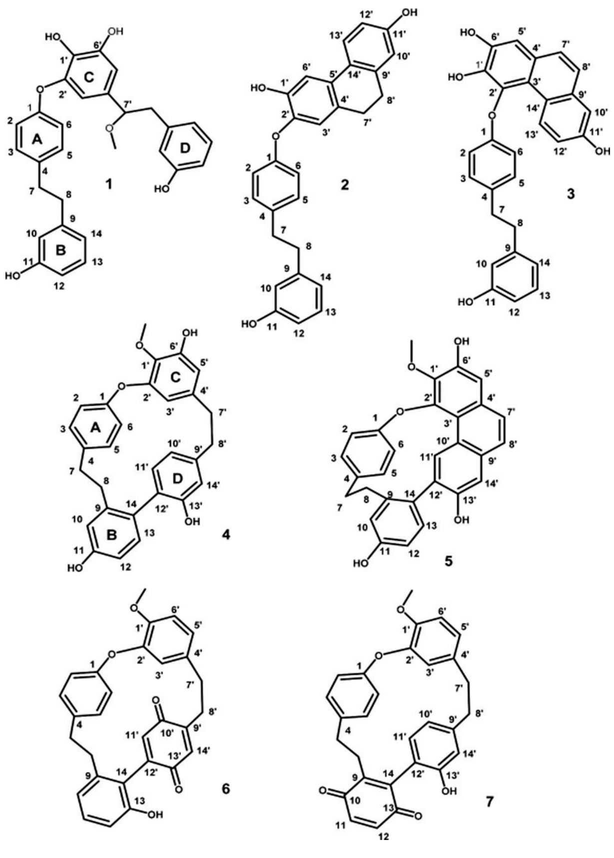

Fig. 4. Structures of seven new bisbibenzyls isolated from the methanol extract of the liverwort *Lunularia cruciata* (Novaković et al., 2019).

Phytochemical research of the bryophytes

Together with the ecophysiological studies, phytochemical studies of bryophytes have also been done. Using the UHPLC/-HESI-MS/MS methods, phenolic compounds were analyzed in *Physcomitrella patens* (Vujičić, 2016). In plants exposed to the short-term stress compared to plants exposed to the long-term salt stress, a significantly greater variety of compounds was observed. The predominantly detected compound was *p*-coumarin acid, which was detected in all treatments except in the control group. Qualitative analysis of phenolic compounds in the plants exposed to the long-term stress showed that the number of detected phenolic compound decreases with the increase in salt concentration. In all tested species dominant phenolic compound was *p*-coumaric acid.

The chemical composition has been investigated on selected moss species: *Rodobrium ontariense, Atrichum undulatum, Hypnum andoi* (Pejin et al., 2012a, 2012c). However, there are more studies of the chemical composition of liverworts. Several liverwort taxa have been the subject of a doctoral dissertation: "Chemical composition and antimicrobial activity of various liverwort extracts of *Scapania aspera* M. & H. Bernet, *S. nemorea* (L.) Grolle, *Porella cordaeana* (Hub.) Moore and *P. arboris-vitae* (With.) Grolle)" (Bukvički, 2014). Analysis of the volatile compounds of different extracts identified 105 compounds in *Scapania aspera* (Bukvicki et al., 2013), 79 in *Scapania nemorea* (Bukvički et al., 2014), 84 in *Porella cordaeana* (Bukvički et al., 2012a)., and 66 in *P. arboris-vitae* (Tyagi et al., 2013). Chemical analysis of the volatile compounds of the extracts revealed the presence of large amounts of sesquiterpene and monoterpenes, and a much lower percentage of hydrocarbons, alcohols, aldehydes and ketones. GC-MS analysis of the hexane solution of methanolic extract of *Lophocolea bidentata* showed two major compounds - two sesquiterpene lactones. Diplophyllolide (eudesmanic-type of sesquiterpene lactone), an important chemotaxonomic marker for Marchantiophyta, and 5-hydroxydiplophyllolide have also been identified. Bis-bibenzyls are a significant group of chemical compounds in liverworts. Methanolic extract of *Marchantia polymorpha* subsp. *ruderalis* contains bisbibenzyl marchantin A as a

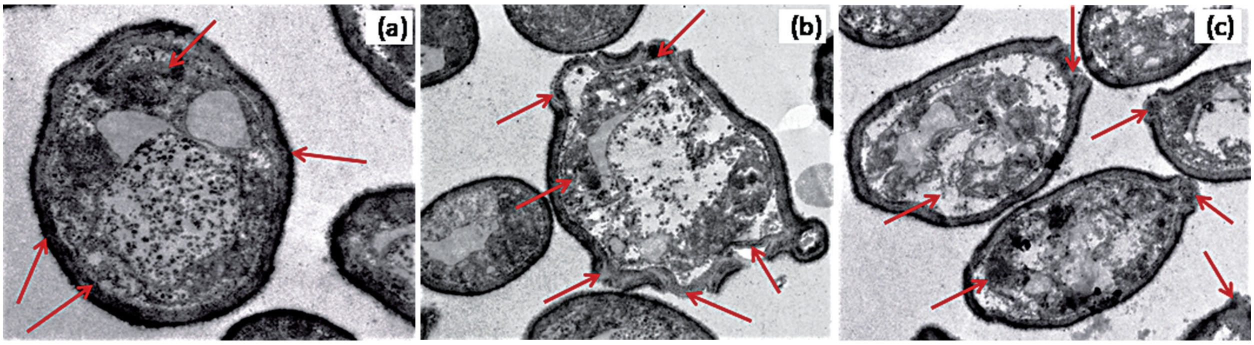

Fig. 5. Transmission electron micrographs of untreated and treated *Salmonella enteritidis* cells. (a) Untreated cells, (b) Methanolic *P. arboris-vitae* extract, and (c) Ethanolic *P. arboris-vitae* extract (Tyagi, 2013)

major component (Sabovljevic et al., 2017). Seven new bis-bibenzyls were isolated from the methanol extract of the liverwort *Lunularia cruciata* (**Fig. 4**) along with one previously known bibenzyl and five known bisbibenzyls (Novaković et al., 2019). These are acyclic bisbibenzyls, perrotetins and cyclic analogues, ricardines.

Recently, microbial transformation of molecules isolated from mosses has been the subject of the investigation. Biotransformation represents an important strategy in the structural modification of natural compounds by microorganisms.

Biological activity of bryophytes

Secondary metabolites of bryophytes have important antibacterial and antifungal potential. This has been confirmed by a series of studies on both mosses and liverworts. Among mosses, following species were studied: *Bryum argenteum* (Sabovljevic et al., 2006), *Pleurozium schreberi, Palustriella commutata, Homalothecium philippeanum, Anomodon attenuatus, Rhytidium rugosum, Hylocomium splendens, Dicranum scoparium, Leucobryum glaucum* (Veljić et al., 2008), *Fontinalis antipyretica, Hypnum cupressiforme, Ctenidium molluscum* (Veljić et al., 2009), *Abietinella abietina, Neckera crispa, Platyhypnidium riparoides, Cratoneuron filicinum, Campylium protensum* (Bukvički et al., 2012b) and *Rhodobryum ontariense* (Pejin et al., 2012b). In general, all extracts showed stronger antifungal than antibacterial potential. However, significantly higher antimicrobial potential has been shown by secondary metabolites in liverworts. In addition to the species from *Scapania* and *Porella* genera that were already mentioned (Bukvički, 2014), *Ptilidium pulcherrimum* was investigated as well (Veljić et al., 2010).

TEM analysis was performed to confirm antimicrobial activity and the mode of action. Transmission electron micrographs of *Salmonella enteritidis* (**Fig. 5**) show the effect of the extracts: (a) control cells; (b) bacterial cell after the treatment with methanolic extract and (c) bacterial cell after the treatment of ethanol extract of the liverwort *P. arboris-vitae* (**Fig. 5**).

More recently, the cytotoxic potential of various molecules (like terpenes, flavonoids, sterols, coumarins, and interesting macrocyclic compounds - bibenzyls and bis-bibenzyls) present mainly in Marchantiophyta, have also been investigated. The cytotoxic activity of the newly isolated compounds, as well as the already known ricardin G was investigated. The isolated compounds showed cytotoxic activity against A549 lung cancer cells with IC50 values of 5.0, 5.0 and 2.5 mM.

Pinguisane-type of compounds isolated from *Porella cordaeana* showed imunomodulatory activity (Radulović et al., 2016).

This chronological overview gives a partial review of bryological researches in Serbia, but also shows endless directions and possibilities of bryology. Bryology has large potential and perspective, considering that every new initiative in bryophyte research has given many internationally significant results. At this point, perhaps main problem in the further development of bryology is the lack of interested researchers.

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