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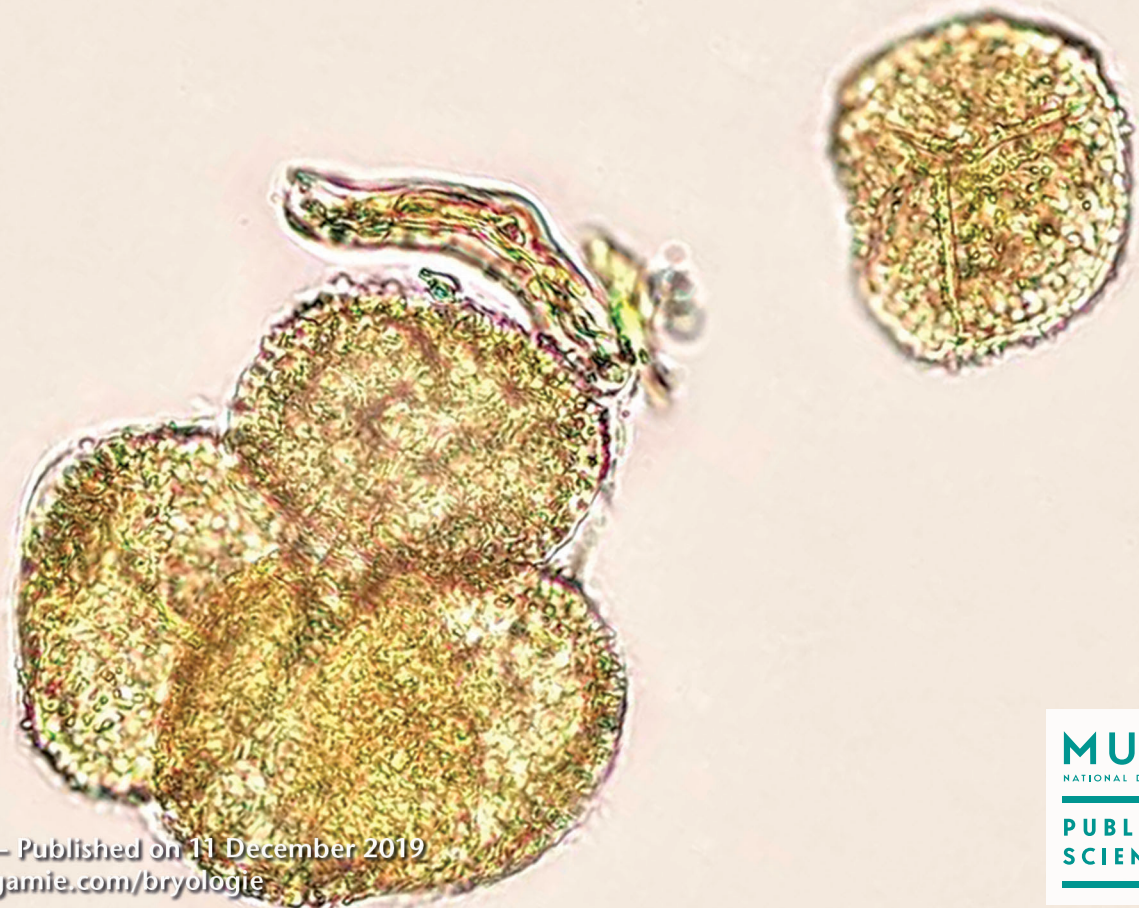
## *Bryologie*

2019 • 40 • 22

### Novelties in the hornwort flora of Croatia and Southeast Europe



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- Publications bibliographiques du CNRS (Pascal).

*Cryptogamie, Bryologie* est distribué en version électronique par / *Cryptogamie, Bryologie is distributed electronically by*:

- BioOne® (<http://www.bioone.org>)

**Cryptogamie, Bryologie** est une revue en flux continu publiée par les Publications scientifiques du Muséum, Paris

*Cryptogamie, Bryologie is a fast track journal published by the Museum Science Press, Paris*

Les Publications scientifiques du Muséum publient aussi / *The Museum Science Press also publish*:

*Adansonia, Geodiversitas, Zoosystema, Anthropolzoologica, European Journal of Taxonomy, Naturae, Cryptogamie* sous-sections *Algologie, Mycologie*.

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[diff.pub@mnhn.fr](mailto:diff.pub@mnhn.fr) / <http://sciencepress.mnhn.fr>

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ISSN (imprimé / *print*): 1290-0796 / ISSN (électronique / *electronic*): 1776-0992

# Novelties in the hornwort flora of Croatia and Southeast Europe

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Submitted on 27 March 2019 | Accepted on 21 August 2019 | Published on 11 December 2019

Rimac A., Šegota V., Alegro A., Koletić N. & Vuković N. 2019. — Novelties in the hornwort flora of Croatia and Southeast Europe. *Cryptogamie, Bryologie* 40 (22): 289-295. <https://doi.org/10.5252/cryptogamie-bryologie2019v40a22>. <http://cryptogamie.com/bryologie/40/22>

## ABSTRACT

Hornworts are globally the least diverse bryophyte group with respect to species number, with only eight species known from Europe. As for Croatia, only two species are listed in the available literature, with most recent record being more than 85 years old. Our study carried out in Central Croatia identified three new species for the hornwort flora, including two new genera – *Notothylas* Sull. ex. A. Gray and *Anthoceros* L. Our finding of very rare Natura 2000 species *Notothylas orbicularis* (Schwein.) A. Gray, reported hitherto only from four Central European countries, is the first in Southeast Europe and the southernmost in Europe. Furthermore, we recorded *Anthoceros agrestis* Paton and *Phaeoceros carolinianus* (Michx.) Prosk., which are either very rare or, more likely, overlooked and understudied in Southeast Europe.

## RÉSUMÉ

*Anthocérotes nouvelles pour la flore de Croatie et du sud-est de l'Europe.*

Dans le groupe des bryophytes, les anthocérotes sont les moins nombreuses en terme de nombre d'espèces; huit espèces seulement sont répertoriées en Europe. Pour la Croatie, seules deux espèces sont mentionnées dans la littérature disponible, l'observation la plus récente date de plus de 85 ans. Notre étude en Croatie centrale a permis d'identifier trois nouvelles espèces pour la flore des anthocérotes, dont deux nouveaux genres – *Notothylas* Sull. ex. A. Gray et *Anthoceros* L. C'est la première fois que la très rare *Notothylas orbicularis*, espèce Natura 2000, est signalée dans le sud-est de l'Europe et il s'agit de sa station européenne la plus méridionale. Jusqu'à présent, elle était signalée dans quatre pays d'Europe centrale. En outre, nous avons observé *Anthoceros agrestis* Paton et *Phaeoceros carolinianus* (Michx.) Prosk., qui sont très rares ou plus probablement négligées et sous-étudiées dans le sud-est de l'Europe.

## KEY WORDS

*Anthoceros agrestis*,  
*Notothylas orbicularis*,  
*Phaeoceros carolinianus*,  
rare species,  
Southeast Europe,  
Croatia,  
new records.

## MOTS CLÉS

*Anthoceros agrestis*,  
*Notothylas orbicularis*,  
*Phaeoceros carolinianus*,  
espèces rares,  
Europe du sud-est,  
Croatie,  
signalements nouveaux.



## INTRODUCTION

Hornworts are the smallest bryophyte group with respect to species diversity, with an estimated 200–250 species worldwide (Villarreal *et al.* 2010), which is quite low in comparison to the liverworts and mosses, which have 7000–9000 (Konrat *et al.* 2010; Söderström *et al.* 2016) and 11 000–13 000, respectively (Magill 2010; Söderström *et al.* 2016). Considering hornwort species diversity across the world, the tropics are the richest, particularly tropical Asia (60 spp.), the Indian subcontinent (58 spp.) and the Neotropics (49 spp.) (Villarreal *et al.* 2010). By contrast, only four genera with altogether eight species have been recognised in Europe so far – *Anthoceros* L. with four species, *Phaeoceros* Prosk. with two and *Notothylas* Sull. ex. A. Gray and *Phymatoceros* Stotler, W. T. Doyle & Crand.-Stotl. with one species each (Hodgetts 2015).

From Southeast Europe, only five species from three genera have been reported – *Anthoceros agrestis* Paton, *A. punctatus* L., *Phaeoceros laevis* (L.) Prosk., *P. carolinianus* (Michx.) Prosk. and *Phymatoceros bulbiculosus* (Brot.) Stotler, W. T. Doyle & Crand.-Stotl. (Sabovljević & Natcheva 2006; Hodgetts 2015).

As for Croatia, data on hornwort diversity can be obtained from several checklists (Pavletić 1955; Düll 1999; Sabovljević 2003; Sabovljević & Natcheva 2006; Ros *et al.* 2007; Hodgetts 2015; Alegro & Šegota 2019), all of them listing only two species – *Phaeoceros laevis* and *Phymatoceros bulbiculosus*. These lists are compilations of old literature data, while no recent records of these species have been made in Croatia. To a considerable extent, this is a result of insufficient research into the bryophytes of Croatia and Southeast Europe, areas that are recognized as less investigated than other parts of Europe (Sabovljević *et al.* 2011). Although significant progress has been made during the past decade (e.g. Papp & Sabovljević 2009; Papp *et al.* 2013a, b, c; Alegro *et al.* 2014, 2015, 2019), continuous reporting of new species indicates that knowledge of bryophyte flora is still insufficient, highlighting the need for additional field surveys in Croatia. Regarding the hornworts, not one record was made in Croatia for more than 85 years, while their occurrence was documented on altogether four occasions (Klinggräff 1861–1862; Heinz 1888; Schiffner 1916; Horvat 1932).

Hornworts are quite inconspicuous ephemeral bryophytes mostly inhabiting transient but regularly recurrent habitats subjected to yearly disturbances and providing sufficiently moist, open and disturbed ground, as this enables the development of ephemerals before the colonization of vascular plants and other mosses takes place (Ahrens 2005; Koval & Zmrhalová 2010; Zechmeister *et al.* 2017). Hornworts display an annual-shuttle life strategy, with a very short lifespan, high sexual reproduction effort and production of large spores. The latter feature does not allow very efficient long distance dispersal into more suitable habitats, but spore longevity does ensure the build-up of a persistent diaspore bank promoting the intermittent occurrence of hornworts whenever favourable environmental conditions occur (Dur-ing 1979; Bisang 1996; Bisang *et al.* 2009).

## MATERIAL AND METHODS

The field study was performed in April 2013 and August 2018 in the surroundings of Nebojan Village in the central part of Croatia. The area is rural with traditional households, hay meadows and arable land dominating the landscape. The climate in the area is temperate humid with warm summers (Filipčić 1998; Šegota & Filipčić 2003). Annual mean air temperature is 11°C, while mean air temperatures in January and August are 0.1 and 21.5°C, respectively. Annual precipitation in the area is 907.5 mm, ranging from 79.2 to 95.1 mm during the summer months (means 1949–2017, Croatian Meteorological and Hydrological Service). Regarding the pedology of the area, the most common soil types are clayey-loamy and loamy soils.

During the study, the habitats suitable for hornwort species were inspected and a list of bryophytes was made at localities that housed the hornworts. Furthermore, available data on hornworts from the literature and herbaria were examined. The nomenclature of bryophytes follows Ros *et al.* (2007, 2013). Collected specimens were deposited in herbarium ZA (Thiers 2019).

## RESULTS AND DISCUSSION

Identification of collected hornwort specimens revealed three new national records, increasing the richness of Croatian bryophyte flora to 711 taxa – 544 mosses, 162 liverworts and five hornworts. The newly recorded species are described in detail below, while their distribution is shown in Fig. 1.

*Anthoceros agrestis* Paton

SPECIMEN EXAMINED. — **Croatia.** Nebojan Village, open ground in a hazelnut plantation, 45.4818°N, 16.1976°E, 190 m a.s.l., 20.IV.2013, *leg.* Rimac A., det. Rimac A. & Šegota V., ZA[ZA49732]; *Ibidem*, open ground on the lawn in a yard, 45.4959°N, 16.2038°E, 176 m a.s.l., 7.VIII.2018, *leg.* Rimac A., det. Rimac A. & Šegota V., ZA[ZA49733]

## SPECIES ACCOUNT

*Anthoceros agrestis* is a monoecious, summer annual species that forms rosettes (Fig. 2A) on open and disturbed, moist or damp soil—mostly clay, loam and loamy-sand. It grows in arable and stubble fields, fallow land, in gardens, marshy pastures, ditch sides, by paths, on river borders and forest edges (Smith 1996; Paton 1999; Dierßen 2001; Atherton *et al.* 2010). The species is a European temperate element (Hill & Preston 1998), with a discontinuous circumpolar distribution, but is also present in North Africa and Macaronesia (Hill & Preston 1998; Dierßen 2001). Although of wide distribution, it is uncommon and sparse and possibly overlooked or misidentified as the more frequently reported *A. punctatus*, from which it is distinguished according to the size of the antheridia (Fig. 2B) (Paton 1999). *Anthoceros agrestis* is commonly associated with *Riccia* L., *Fossombronina* Raddi and *Phaeoceros*

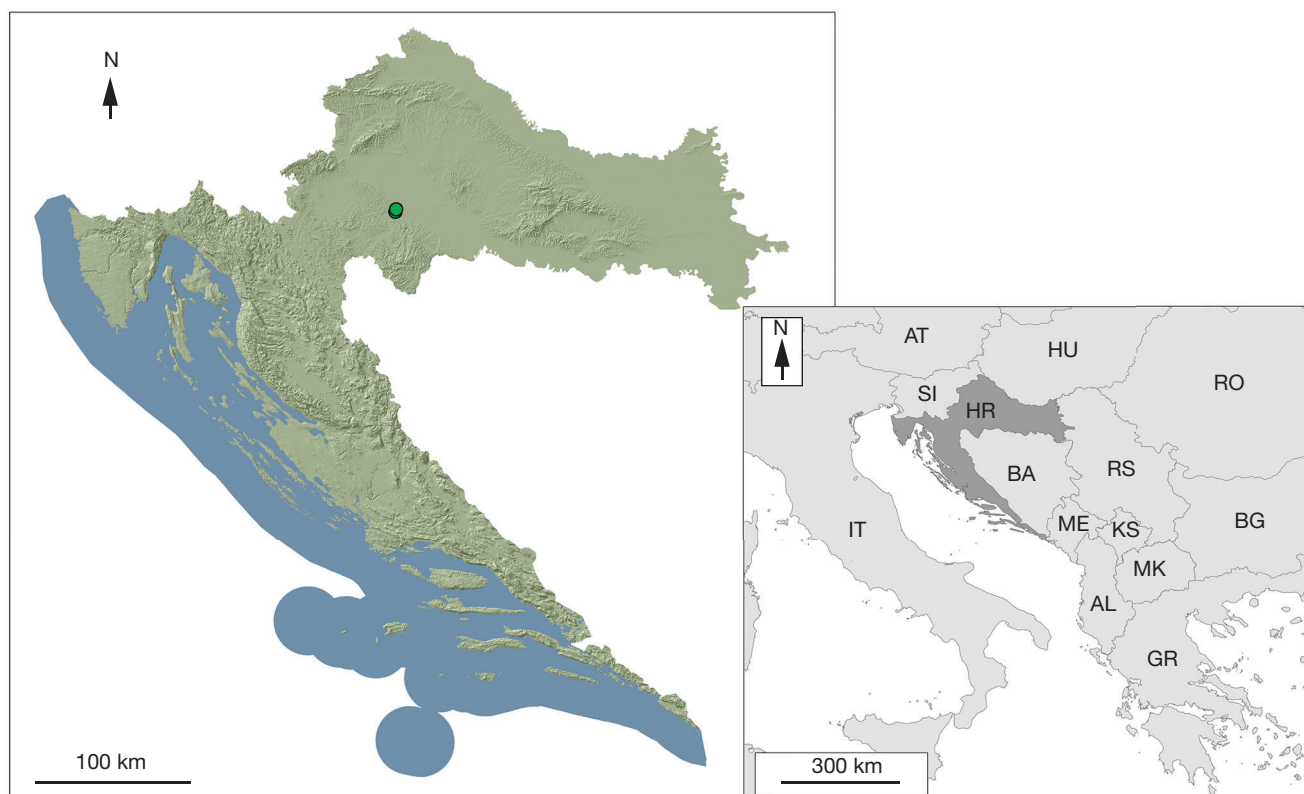


FIG. 1. — Occurrence sites of new hornwort species in Croatia. The map inserted bottom right shows the position of Croatia in SE Europe.

species in the plant community *Riccio glaucae-Anthocerotetum* (Dierßen 2001). The species is present throughout Europe (Hodgetts 2015). In the Mediterranean part, it has been reported from the Iberian and the Apennine Peninsula and from large islands – Crete, Sardinia, Sicily, the Canary Islands and Madeira (Ros *et al.* 2007; Hodgetts 2015). However, it is relatively rare or more likely under-recorded in Southeast Europe, where it was reported so far only from Macedonia (Cekova 2005), Crete (Düll 1979), Bulgaria (Sabovljević & Natcheva 2006) and Romania (Ştefănuţ & Goia 2012), in the latter being considered vulnerable (VU) (Ştefănuţ 2014).

### *Notothylas orbicularis* (Schwein.) A. Gray

**SPECIMEN EXAMINED.** — **Croatia.** Nebojan Village, open ground on the lawn in a yard, 45.4959°N, 16.2038°E, 176 m a.s.l., 7.VIII.2018, *leg.* Rimac A., det. Rimac A. & Šegota V., ZA[ZA49736].

### SPECIES ACCOUNT

This is a monoecious annual species that grows in small rosettes (Fig. 2G) on open and disturbed, loamy or clayey soil. Preferred habitats are arable fields, particularly stubble, but it can also inhabit open ground on roadsides, alluvial soil on river margins (Schuster 1992; Schröck *et al.* 2014) and was reported from dried out rice fields in Japan (Hasegawa 1979). The species has a cosmopolitan distribution but is overall very rare; distributed throughout the Holarctic region, it is

also present in tropical Africa, South and Central America (Dierßen 2001) and has recently been documented in Australia (Cargill 2016). The species has been reported from only four European countries and is limited to Central Europe (Jack 1898; Saukel & Köckinger 1999; Kučera *et al.* 2012; Ludwig *et al.* 1996; Moose Deutschland 2019). Namely, the species has been recently reported in the Austrian states Styria, Upper and Lower Austria and Carinthia (Schröck *et al.* 2014; Köckinger 2017; Zechmeister *et al.* 2017) and in the German states Hessen (Manzke & Wentzel 2003; Manzke 2006) and Rhineland-Palatinate (Fischer *et al.* 2008), while only old historical data exist for the regions of Saxony (Schade 1924) and Bavaria (Paul 1943). In the Czech Republic, it was rediscovered in 2010 after more than 90 years (Duda 1972) in four new localities, where only seven individuals, accompanied by the also very rare *Anthoceros neesii* and the somewhat more frequent *Anthoceros agrestis*, were recorded on moist edges of stubble fields in the northeastern part of the country (Koval & Zmrhalová 2010). Finally, a historical record of *N. orbicularis* dates back to 1898, for Trentino-Alto Adige region in northern Italy (Jack 1898), but the species was never found again in the country (Aleffi *et al.* 2008).

Having in mind the species distribution in Europe, the finding of *N. orbicularis* in Croatia is an especially valuable contribution to the bryophyte flora of the country, Southeast Europe and Europe as a whole. It is the first report of the species outside Central Europe, being the southernmost finding of this rare species in Europe.

The species is threatened with extinction in Austria, as stated in Köckinger (2017), but considered critically endangered in the country according to Hodgetts (2015). It is critically endangered (CR) in the Czech Republic (Kučera *et al.* 2012), endangered (EN) in Germany (Ludwig *et al.* 1996) and data deficient (DD) in Italy (Hodgetts 2015). It is included in the Red Data Book of European Bryophytes (ECCB 1995) and listed on Annex II (animal and plant species of Community interest whose conservation requires the designation of special areas of conservation) of EU Habitats Directive (European Community 1992).

### *Phaeoceros carolinianus* (Michx.) Prosk.

SPECIMEN EXAMINED. — Croatia. Nebojan Village, open ground on the lawn in a yard, 45.4959°N, 16.2038°E, 176 m a.s.l., 7.VIII.2018, leg. Rimac A., det. Rimac A. & Šegota V., ZA[ZA49735].

### SPECIES ACCOUNT

The species is a summer annual, inhabiting a similar set of habitats as the above described *Anthoceros agrestis* and *Notothylas orbicularis*, arable fields being the most common (Paton 1999; Atherton *et al.* 2010). The species is monoecious, in contrast to a very similar dioecious *P. laevis*, for which it can easily be misidentified.

*Phaeoceros carolinianus* (Fig. 2D) is a European temperate element with a discontinuously circumpolar distribution, occurring also in the southern Hemisphere (Hill & Preston 1998) – Australia (McCarthy 2003), New Zealand (Glenny 1998), Africa (Fischer 2007; Wigginton 2009) and South America (Hässel de Menéndez & Rubies 2009). The species has been reported from the majority of central, northern and western European countries (Hodgetts 2015). In Mediterranean Europe, it is present in the Iberian but absent from the Apennine Peninsula and larger islands (Ros *et al.* 2007). In southeast Europe, *P. carolinianus* has been reported from three countries – Romania (Ștefănuț & Goia 2012), Slovenia (Martinčič 2016) and Bulgaria (Ganeva & Natcheva 2003). It is treated as critically endangered (CR) in Romania (Ștefănuț & Goia 2012), data deficient (DD) in Bulgaria and Slovenia (Hodgetts 2015; Martinčič 2016).

### HABITATS AND THREATS

During our research, hornworts were found growing on patches of open ground in a hazelnut plantation and in a yard of a rural house. Interestingly, in the latter locality some of the open loamy soil patches were molehills, from which the excess soil had been removed, the rest compacted during lawn maintenance. In this locality, the hornwort flora was abundantly developed in early August. Fertile *Anthoceros agrestis* (Fig. 2A–C) and *Phaeoceros carolinianus* (Fig. 2D–F) individuals were the most conspicuous with plentiful ripe sporophytes, while the presence of *Notothylas orbicularis* (Fig. 2F–H) was revealed only after the collected material was examined under stereomicroscope so that the collection turned out to be truly serendipitous. Only one fertile thallus, 7 mm in diameter, with

several ripe sporophytes was gathered, making the identification possible (Fig. 2F). Hornworts were here accompanied by *Riccia glauca* L., *Physcomitrella patens* (Hedw.) Bruch & Schimp., *Tortula truncata* (Hedw.) Mitt. and *Marchantia polymorpha* L., forming the plant community *Riccio glaucae-Anthocerotetum crispuli* Koppe ex Neum., 1971, belonging to the alliance *Phascion cuspidatae* Waldh. ex v. Krus., 1945, a euhemerophilous pioneer bryophyte vegetation on temporary dry and dry loamy soils in the nemoral and boreal zones (Mucina *et al.* 2016). In the hazelnut plantation, *Anthoceros agrestis* was fructifying as early as late April, which is earlier than reported from Central European localities, where this species completes its life cycle from germination to sporophyte development and spore maturation during the late summer and autumn (Bisang 1995, 2004; Zechmeister *et al.* 2017). In this locality, *Anthoceros agrestis* was associated with *Riccia nigrella* DC. and *Tortula truncata*, forming the same community as in the previous locality.

In central Europe, these hornwort species grow mainly in arable fields, where farming routines and crop type are the most important factors determining their occurrence (Bisang 1998; Bisang *et al.* 2009). The most suitable habitats are cereal stubble fields and bare fields that are left unmanaged until the late summer or the following spring, which enables the completion of the whole life cycle before the tillage (Bisang 1998; Bisang *et al.* 2009). Accordingly, the decline in hornwort populations observed during the last few decades in Europe was attributed primarily to intensive agricultural practices (Bisang 1992, 1999; Ahrens 2005), to earlier tillage operations, resulting in a reduced period between the harvest and tillage, and to a lesser extent to the use of pesticides and fertilizers. These threats are nowadays widely present and pose a threat to the hornworts in Croatia as well, especially in the eastern, predominantly agricultural part of the country. Additionally, the abandonment of agriculture and subsequent succession of arable land into woodland vegetation can be observed to some extent throughout Croatia and represents another serious and increasing threat to the hornworts. Moreover, our knowledge on the distribution of hornwort species is extremely poor so the main requirement for their protection and conservation is not satisfied. With these three novelties from only two close localities (Fig. 1), Croatian hornwort flora now counts five species, while prior to our research, only two species of this peculiar bryophyte group were known from the literature. *Phaeoceros laevis* was recorded on three occasions in the continental part of Croatia: on a sandy substrate along the ditches in the Sava River floodplain (Heinz 1888) in Zagreb, on cultivated soil in the vineyard region in the surroundings of Zagreb (Klinggräff 1861–1862), and, the most recent record, that of Horvat (1932), was from loamy path edges in the Hrvatsko Zagorje Region (northern Croatia). *Phymatoceros bulbiculosus* was recorded only once in the south of the Mediterranean part of the country, on a limestone substrate in the vineyards of Lastovo Island (Schiffner 1916).

Although our findings represent a valuable contribution to the hornwort flora of Croatia, a comprehensive study targeting suitable habitats should be conducted in order to assess horn-



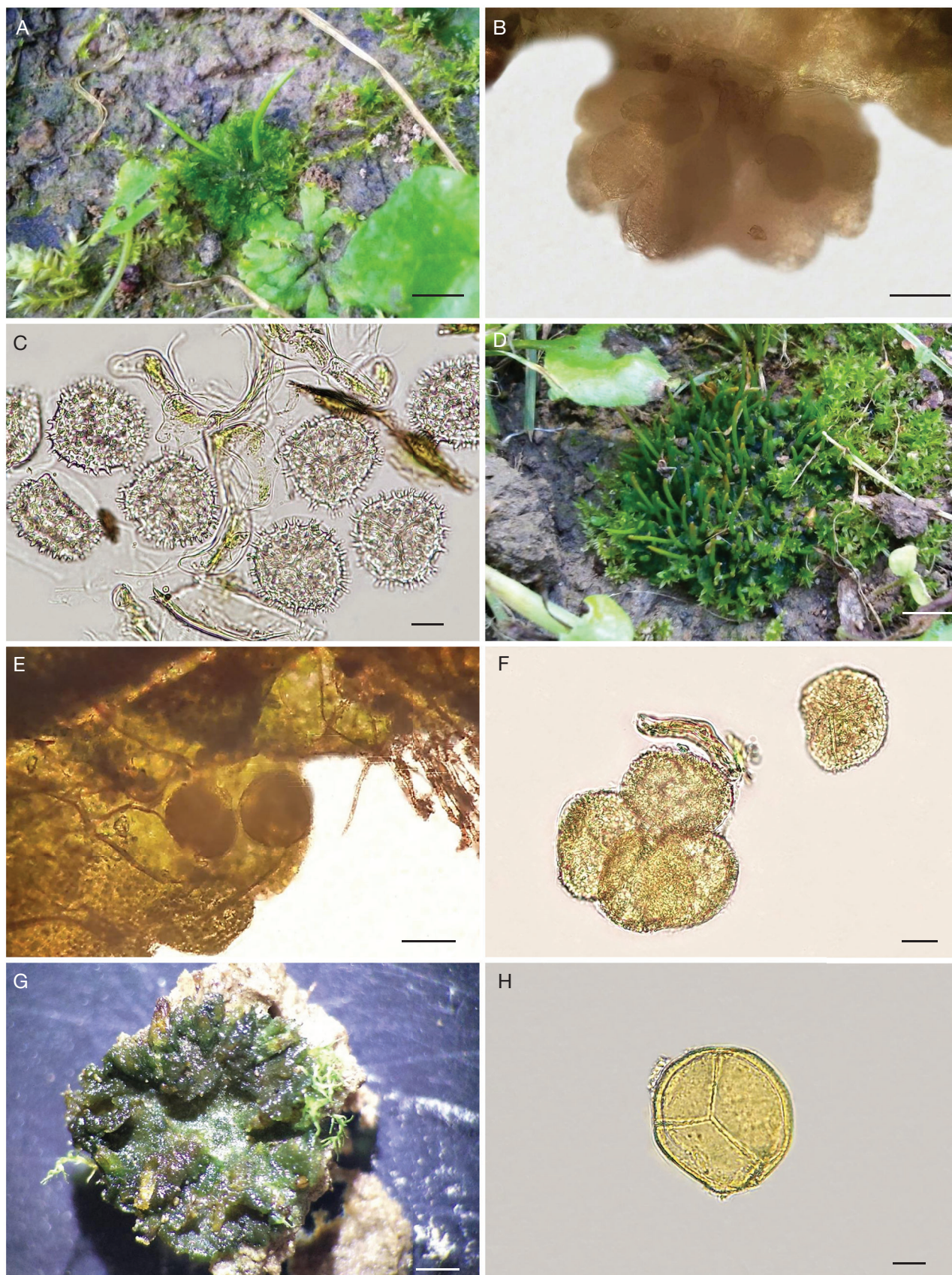


FIG. 2. — *Anthoceros agrestis* Paton: A, thallus with sporophytes; B, antheridia; C, spores with pseudo-elaters; *Phaeoceros carolinianus* (Michx.) Prosk.: D, thallus with sporophytes, E, antheridia; F, spores; *Notothylas orbicularis*: G, thallus with sporophytes, H, spore. Scale bars: A, C, F, 25  $\mu$ m; B, D, 50  $\mu$ m; E, 150  $\mu$ m; G, 1  $\mu$ m; H, 10  $\mu$ m.



wort distribution, diversity, habitat preferences and phenology, which will provide the basis for the eventual protection and monitoring of these species, as well as of their habitats.

## Acknowledgements

We are grateful to two reviewers and the editor, which thoughtful comments substantially improved our manuscript.

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Submitted on 27 March 2019;  
accepted on 21 August 2019;  
published on 11 December 2019.