

**FIRST FINDINGS OF SUBTERRANEAN GAMETOPHYTES OF THE GENUS *DIPHASIASTRUM* IN LITHUANIA**
**Radvilė RIMGAILĖ-VOICIK\*, Jonas Remigijus NAUJALIS**

Vilnius University, Department of Botany and Genetics, M. K. Čiurlionio Str. 21/27, Vilnius LT-03101, Lithuania

\*Corresponding author. E-mail: radvile.rimgailaite@gmail.com

**Abstract**

RIMGAILĖ-VOICIK R., NAUJALIS J. R., 2015: First findings of subterranean gametophytes of the genus *Diphasiastrum* in Lithuania [Pirmieji *Diphasiastrum* genties pataisų požeminių gametofitų radiniai Lietuvoje]. – Both. Lith., 21(2): 133–135.

Gametophytes of the genus *Diphasiastrum* sp. were recorded for the first time in Lithuania. The occurrence rates, morphological features were described, illustrated and discussed. Voucher specimens are deposited at Vilnius University Herbarium (WI).

**Keywords:** club mosses, *Diphasiastrum*, gametophytes, *Lycopodiaceae*.

Archaic homosporous club moss (*Lycopodiaceae* P.Beauv. ex Mirb.) perennial evergreen sporophyte clones in temperate forests can grow up to several meters in length (NAUJALIS, 1995; BENCA, 2014). New club moss sporophytes originate from subterranean, achlorophyllous gametophytes (also called prothallia) that are associated with fungal endophytes (HORN et al., 2013). To our knowledge, there are no published data on dynamics and structure of subterranean gametophyte populations in nature. Studies on subterranean gametophyte populations in nature remain exploratory and reports on finding gametophytes are rare (HORN et al., 2013). Considerably large gametophyte populations in forests are formed from one (BRUCE, 1972; THOMAS, 1975) up to six species (BRUCE & BEITEL, 1979). We suppose that the lack of evidence remains mainly because there is no precise methodology for localization of gametophytes in a habitat.

Gametophytes of modern lycophytes in the temperate climate zone were discovered and described for the first time by FRANKHAUSER (1873). Later, BRUCHMANN (1898) generalized his and other pteridologists' work of that time and described five structural types

of gametophytes, all named by representing species: *Lycopodium clavatum* L. – Type I, *L. complanatum* L. (= *Diphasiastrum complanatum* (L.) Holub) – Type II, *L. selago* L. (= *Huperzia selago* (L.) Bernh. ex Schrank et Mart.) – Type III, *L. inundatum* L. (= *Lycopodiella inundata* (L.) Holub) – Type IV and *L. phlegmaria* L. (= *Huperzia phlegmaria* (L.) Rothm.) – Type V). ROTHMALER (1944) emphasized the taxonomic value of gametophyte morphological features, but later this approach was discarded. Types described by Bruchmann are still used to characterize gametophytes of club mosses (WHITTIER, 1977, 1981, 2003, 2006; RENZAGLIA & WHITTIER, 2013).

Subterranean *Lycopodium* L. sp. (Type I) gametophytes were found for the first time in Lithuania in wet black alder forests; later in the dry pine forests of Švenčionys and Varėna districts (NAUJALIS, 1995). In our study, established in 2012, *Diphasiastrum* Holub sp. (Type II) gametophytes were found for the first time in Lithuania in the pine forest near Senoji Varėna (Varėna district, 54°28'4" N, 24°60'3" E).

In previous studies (NAUJALIS, 1995), sieves were used for gametophyte extraction from soil. That method was not suitable for the following reasons:

1) in many cases gametophytes were grounded with rhizoids in the soil and sieving caused damage; 2) it was not possible to determine distribution and spatial relations between gametophytes; 3) because of the damage caused by sieving the determination of developmental stages was hardly possible, emerged underground sporophytes were broken. Because of the reasons mentioned, new method was applied. Gametophytes were searched in 0.25 m<sup>2</sup> soil samples collected with intact forest floor of 10 cm in depth. The method similar to one used by zoologists for quantifying soil invertebrates (GHILAROV & STRIGANOVA, 1987) was used for the search of gametophytes. In the lab, the moss layer was removed. Then, using pins, every sample was divided into 10 × 10 cm plots to increase accuracy. We looked for gametophytes by gradually disassembling soil samples (NAUJALIS, 1995) with tweezers. The coordinates and soil depth of every gametophyte located was registered. Depth was determined by comparison with the closest intact sample part that had the moss layer removed. A total of 31 soil samples were examined and 596 gametophytes were found. Most of the gametophytes, 524 (87.9%), were of irregular bowl shape (Type I) and 72 (12.1%) were of carrot or beetroot shape (Type II). Type II gametophytes belong to the genus *Diphasiastrum* (Fig. 1). Similarly, Type II gametophytes, the same as Type I, had no fixed external features that would help to identify to which species (*D. complanatum* (L.) Holub., *D. tristachyum* (Pursh) Holub or *D. ×zeilleri* (Rouy) Holub) they belong. Seven out of 72 Type II gametophytes had sporophyte sprout. Eleven juvenile *Diphasiastrum* Holub. sporophytes without remains of gametophytes were also found.



Fig. 1. *Diphasiastrum* sp. (Type II) subterranean gametophytes

All gametophytes found were in humus soil horizon. Upper part of gametophytes was located in the depth of 0.2 cm, few were found in the depth of 1.2–1.8 cm. Voucher specimens are deposited at Vilnius University Herbarium (WI), sample No. P31378.

Describing external features of gametophytes, we employed BRUCE (1979) terminology. Three typical zones were observed: tapering base, central meristem, gametangial cap. Type II gametophytes were of different ages of maturity as gametangial caps were unevenly developed. In many cases gametangial caps were not expressed, also rhizoids were absent or occurred singly. The shape of Type II gametophytes varied from tear drop to carrot-beetroot shape. Gametophyte lower parts were angled off to right or left. Possibly the reason of flexion was obstacles in the soil. WHITTIER (1981) noticed that flexion can occur because of anthridiogen conglomeration. BRUCHMANN (1898) indicated that Type II gametophytes were 8 mm in length and 4 mm in width; the largest gametophytes were 12 mm in length and 5 mm in width and weighted 0.15 g. Size parameters determined by us were slightly different. Average length of gametophytes found by us was  $9.4 \pm 2.3$  mm, width average was  $3.7 \pm 0.9$  mm; the maximum length determined was 13.4 mm, the maximum width was 5.9 mm.

New genus *Diphasiastrum* sporophytes occur from subterranean achlorophyllous gametophytes, which emerge from spores germinated in the soil. To date, a serious lack of information about *Diphasiastrum* gametophytes and their population functioning remains. No accurate methodology for localizing gametophytes has been developed yet. We located gametophyte populations according to the presence of juvenile sporophytes. The search for juvenile club moss populations in forests is time consuming. Determination of specific microhabitat conditions would help to increase probability to find club moss gametophyte populations.

During the research conducted in 2012–2014, almost 88% of gametophytes found in humus horizon represented the genus *Lycopodium* (Type I) and only 12% represented the genus *Diphasiastrum* (Type II). Sparse Type II gametophyte occurrence rate possibly show lower spore viability and gametophyte fertilization rate in *Diphasiastrum* compared to *Lycopodium*. Type II gametophytes were found for the first time in Lithuania.

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## PIRMIEJI *DIPHASIASTRUM* GENTIES PATAISŲ POŽEMINIŲ GAMETOFITŲ RADINIAI LIETUVOJE

Radvilė RIMGAILĖ-VOICIK, Jonas Remigijus NAUJALIS

### Santrauka

*Diphasiastrum* sp. (padraikų) genties gametofitai Lietuvoje buvo rasti pirmą kartą. Straipsnyje aptariamos potencialių gametofitų augimo vietų nustatymo bei jų išrinkimo iš dirvožemio problemos, iliustruota

ir aprašyta šių gametofitų morfologija bei pasitaikymo dažnis Pietryčių Lietuvos pušynuose. Gametofitų pavyzdžiai saugomi Vilniaus universiteto Herbariume (WI).