The Genus *Diphasiastrum* and Its Lycopodium Alkaloids*

Authors Elsa Steinunn Halldorsdottir, Natalia Magdalena Kowal, Elin Soffia Olafsdottir

Affiliation Faculty of Pharmaceutical Sciences, School of Health Sciences, University of Iceland Hagi, Reykjavik, Iceland

Key words
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- Lycopodiaceae
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- lycopodium alkaloids

Abstract

The genus *Diphasiastrum* includes at least 23 species distributed primarily across the northern temperate and subarctic areas of the world. These plants produce an array of lycopodium alkaloids, and some species such as *Diphasiastrum complanatum* have been used in traditional medicine for ages for various conditions. Hybridization is common in this group of plants and they have always been a challenge for taxonomists and other scientists studying them. To date, 11 *Diphasiastrum* species have been reported to produce lycopodium alkaloids. In this review, reported alkaloids and their distribution patterns across these species along with taxonomical and bioactivity considerations are reviewed and discussed.

Introduction

When we began to study the alkaloid content of *Diphasiastrum alpinum* (L.) Holub (Lycopodiaceae), the only *Diphasiastrum* species growing in Iceland [1], we discovered that the current knowledge on the status of the chemistry and taxonomy of this genus in the literature was rather spread, disordered, and confusing. Even the existence of this group of plants as a separate genus was still under debate. We found that a comprehensive review including discussions on taxonomic status and the known alkaloid contents of species investigated would be very helpful for future studies of this genus. Our aim is to contribute to this matter with the following review.

Club Mosses

Evolution

Club mosses belong to the plant order Lycopodiales. They are spore forming, slow-growing vascular plants dating back to the late Silurian geological period about 300–400 million years ago. Fossil records show that they lived amongst the earliest known land plants and contributed to a large part of the vegetation on Earth in pre-angiosperm times [2–4]. Although many species and groups of club mosses are now extinct, a small part of them has survived. Some species of *Huperzia* club mosses have been called "living fossils" because they have very similar morphological characters to their fossil relatives that lived millions of years ago [2]. This indicates that their genome has not changed much through this long period of vast biological evolution. Along this line, Wagner and Beitel stated: "The Lycopodiaceae as we know them are diverse modern survivors of an ancient lineage" [5]. Club mosses are incredibly effective chemical factories and produce an array of secondary metabolites called lycopodium alkaloids [6–8]. It is fascinating to imagine that maybe these ancient plants were producing the same or similar alkaloids already very early in the evolutionary history of terrestrial plants, and that these compounds might have contributed to their survival.

Medical uses

Club mosses have been used in traditional medicine for centuries and have been valuable herbal medicines in different ethnic societies around the world. The application of club moss spores from, e.g., *Lycopodium clavatum* L. (Lycopodiaceae) or *Diphasiastrum complanatum* (L.) Holub directly to wounds and rashes is well known from natives in North America and Europe [8]. In Ice-

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Bibliography
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Correspondence
Prof. Dr. Elin Soffia Olafsdottir
Faculty of Pharmaceutical Sciences
School of Health Sciences
University of Iceland Hagi
Hofsvallagata 53
107 Reykjavik
Iceland
Phone: + 354 525 5804
Fax: + 354 525 4071
elinsol@hi.is

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Table 1 List of names and synonyms of all *Diphasiastrum* species. The six species marked in bold are found in Europe.

Generally accepted names	Synonym
***D. alpinum* (L.) Holub**	*D. complanatum* ssp. *alpinum* (L.) Jermy, *Di. alpinum* (L.) Rothm, *L. alpinum* L.
D. angustiramosum (Alderw.) Holub	*L. complanatum* var. *angustiramosum* Alderw.
D. carolinum (Lawalrée) Holub	*Di. carolinum* Lawalrée
***D. complanatum* (L.) Holub**	*Di. anceps* Á. Löve & D. Löve, *Di. complanatum* (L.) Rothm., *Di. wallrothii* H. P. Fuchs, *L. complanatum* L.
ssp. *complanatum*	*L. complanatum* ssp. *ancpes* (Wallr.) Milde, *L. complanatum* ssp. *moniliforme* Lindm.
ssp. *montellii* (Kukkonen) Kukkonen	*D. montellii* (Kukkonen) Miniaev & Ivaneno, *Di. complanatum* ssp. *montellii* Kukkonen, *L. complanatum* ssp. *montellii* (Kukkonen) Karlsson
D. digitatum (Dill. ex A. Braun) Holub	*L. digitatum* Dill., *L. flabelliforme* (Fernald) Blanch.
D. fawcettii (F. E. Lloyd & Underw.) Holub	*L. fawcettii* F. E. Lloyd & Underw.
D. x *habereri* (House) Holub	*L. habereri* House
D. henryanum (E. D. Br. & F. Br.) Holub	*L. henryanum* E. D. Br. & F. Br.
***D.* x *issleri* (Rouy) Holub**	*Di. hastulatum* Slipliv, *Di. issleri* Holub, *L. alpinum* ssp. *issleri* Chass, *L. complanatum* ssp. *issleri* Domin, *L. issleri* Domin
D. madeirense (J. H. Wilce) Holub	*Di. madeirense* (J. H. Wilce) Rothm., *L. madeirense* J. H. Wilce
D. multispicatum (J. H. Wilce) Holub	*L. multispicatum* J. H. Wilce
D. nikoense (Franch. & Sav.) Holub	*D. sitchense* var. *nikoense* (Franch. & Sav.) Á. Löve & D. Löve, *L. nikoense* Franch. & Sav.
D. novoguineense (Nessel) Holub	*L. alpinum* var. *novoguineense* Nessel, *L. novoguineense* (Nessel) Herter
***D.* x *oellgaardii* (Stoor et al.) B. Bock**	*L. oellgaardii* (Stoor et al.) B. Bock
D. platyrhizoma (J. H. Wilce) Holub	*Di. platyrhizoma* (J. H. Wilce) Rothm, *L. platyrhizoma* J. H. Wilce
D. sabinifolium (Willd.) Holub	*L. sabinifolium* Willd.
D. sitchense (Rupr.) Holub	*Di. sitchense* Á Löve & D. Löve, *L. sitchense* Rupr.
D. thyoides (Humb. & Bonpl. ex. Willd.) Holub	*L. thyoides* Humb. & Bonpl. ex. Willd., *L. complanatum* var. *thyoides* (Humb. & Bonpl. ex. Willd.) Christ
***D. tristachyum* (Pursh) Holub**	*D. complanatum* ssp. *chamaecyparissus* (A. Braun ex Mutel) Kukkonen, *Di. chamaecyparissus* (A. Braun ex Mutel) Á. Löve & D. Löve, *Di. complanatum* ssp. *chamaecyparissus* (A. Braun ex Mutel) Kukkonen, *Di. tristachyum* (Pursh) Rothm., *L. chamaecyparissus* A. Braun ex Mutel, *L. clavatum* var. *tristachyum* (Pursh) Hook, *L. complanatum* ssp. *chamaecyparissus* (A. Braun ex Mutel) Celak., *L. tristachyum* Pursh
D. veitchii (Christ) Holub	*L. veitchii* Christ
D. wightianum (Grev. & Hook.) Holub	*L. wightianum* Grev. & Hook.
D. zanclophyllum (J. H. Wilce) Holub	L. zanclophyllum J. H. Wilce
***D.* x *zeilleri* (Rouy) Holub**	*D. complanatum* ssp. x *zeilleri* (Rouy) Kukkonen, *Di. complanatum* ssp. x *zeilleri* (Rouy) Pacyna, *Di. complanatum* var. *polystachyum* (H. Lindb.) Kukkonen, *Di.* x *zeilleri* (Rouy) Damboldt, *L. complanatum* ssp. x *zeilleri* (Rouy) Karlsson, *L. complanatum* var. *intermedium* Lindq., *L. complanatum* var. *zeilleri* Rouy, *L.* x *zeilleri* (Rouy) Greuter & Burdet

L. = Lycopodium; D. = Diphasiastrum; Di. = Diphasium

land, *D. alpinum* and *Lycopodium annotinum* L. spores were used for the same purpose and extracts of *L. annotinum* were used for digestive problems, pain, and dysentery [9,10]. Teas of *L. clavatum, D. complanatum*, and other club moss species have also been used for a variety of medical conditions including inflammation, kidney and bladder symptoms, infections and skin diseases, and neurological disorders [11–13]. *Diphasiastrum thyoides* (Humb. & Bonpl. ex Willd.) Holub is used by the Quechua ethnic group in Ecuador to treat disorders of childbirth and as medicine for CNS-related conditions [14]. In China, club mosses have been used for bruises, strains, swellings, neurological disorders such as schizophrenia and for the neurodegenerative diseases Myasthenia gravis and Alzheimer's. A Chinese herbal mixture named Shi Song is described in old pharmacopeias and contains several species of Lycopodiaceae including *Huperzia serrata* (Thunb. ex Murray) Trevis., *Lycopodium japonicum* Thunb. ex Murray, *L. annotinum, Lycopodium obscurum* L., and *D. complanatum* [15,16]. After the discovery of the acetylcholinesterase (AChE) inhibitor huperzine A from *H. serrata*, this herb has become a popular dietary supplement in China and the USA and is promoted as a treatment for Alzheimer's [16].

Classification

There has been an ongoing debate concerning the taxonomy and nomenclature of the plant order Lycopodiales [7]. Four main key systems have been suggested: Wagner & Beitel [5], Holub [17], Öllgaard [18], and Ching [19]. The systems differ in classification into genera, families, subfamilies, and number of species and subspecies. Up to 11 genera have been suggested for the Lycopodiaceae [17], and *Diphasiastrum* plants have been classified as a separate genus or as a part of the *Lycopodium* genus. Furthermore, some have suggested a separate family of Huperziaceae for the *Huperzia* genus [7,17,19]. Today, the classification of the *Diphasiastrum* species to a separate genus is generally recognized, and most European taxonomists support the maintenance of one family of Lycopodiaceae including the four major genera: *Lycopodium, Diphasiastrum, Huperzia*, and *Lycopodiella* [20–23]. In this review we will focus on the genus *Diphasiastrum* and its alkaloid content.

Diphasiastrum genus

The genus *Diphasiastrum* is considered the taxonomically most complex group within the Lycopodiaceae [18,24]. Approximately 25 species can be distinguished and differ morphologically from the closely related *Lycopodium* species [21,25,26]. **Table 1** includes 23 species of the genus *Diphasiastrum*, all described by Holub in 1975 [21] except for *Diphasiastrum* x *ollegaardii* (Stoor

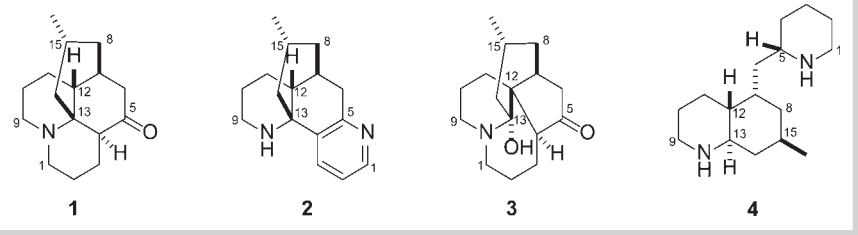


Fig. 1 Representatives of the four structural groups of lycopodium alkaloids: lycopodine (**1**), lycodine (**2**), fawcettimine (**3**), and phlegmarine (**4**).

et al.) B. Bock [27]. Hybridization, where different species parent a new fertile hybrid, is remarkably common amongst the *Diphasiastrum* plants, and known hybrids are treated as "good species" [24,26]. DNA analytical techniques have been used to study hybridization and polyploidy in the *Diphasiastrum* genus [24,26] and the phylogenic relationships have been studied by Aagaard et al. [28]. The debate on the taxonomy of the club mosses discussed above is reflected in an abundance of synonyms for the *Diphasiastrum* species as shown in **Table 1**. This is important to be aware of when studying the literature for these plant species.

Unlike other genera of Lycopodiaceae, *Diphasiastrum* is found mainly in northern temperate and subarctic parts of the world [20], and species which grow at more tropical and subtropical latitudes always grow at high altitudes, such as *Diphasiastrum multispicatum* (J.H. Wilce) Holub, which inhabits the highest mountain peaks of Thailand [25]. In Europe, six *Diphasiastrum* species have been described [29] and they are marked in bold in **Table 1**. The European species are intensively studied with regard to hybridization among related taxa and three of them (marked with an x in their names according to Holub [21]), *Diphasiastrum* x *issleri* (Rouy) Holub (AC hybrid), *D.* x *oellgaardii* (AT hybrid), and *Diphasiastrum* x *zeilleri* (Rouy) Holub (CT hybrid), are hybrids of the parenteral species *D. alpinum* (A), *D. complanatum* (C), and *Diphasiastrum tristachyum* (Pursh) Holub (T) [4,24]. Further hybridization has been described for species of the genus *Diphasiastrum*, especially in "microevolutionary active regions" [24] such as central Europe, making their classification even more complex.

Lycopodium Alkaloids and Their Bioactivity

Lycopodium alkaloids can be divided into four groups. The model compounds for these structural classes [lycopodine (**1**), lycodine (**2**), fawcettimine (**3**), and phlegmarine (**4**)] [7,30] are shown in **Fig. 1**. The total number of reported alkaloids from Lycopodiaceae species, in general, is more than 250 [6–8]. The lycopodane class is the largest group and the most widely distributed, and has been found in more than 30 species of Lycopodiaceae [7]. Lycopodine (**1**) was the first lycopodium alkaloid to be isolated in 1881, and it was indeed from the widely distributed *Diphasiastrum* species *D. complanatum* (syn. *Lycopodium complanatum* L.) [31].

Knowledge of the biological activity of the lycopodium alkaloids is limited, and surprisingly few of the more than 250 reported alkaloids have yet been tested for any kind of bioactivity. A probable reason could be that many Lycopodiaceae plants are slow growing and vulnerable, and often only low quantities of pure alkaloids were isolated. Annotine isolated from *L. annotinum* was shown to affect the maturation of dendritic cells and direct T cells toward a Th2/Treg phenotype in a recent study [32] and huperzine A (**Fig. 2**) has also been shown to affect inflammatory responses [33–37]. The dimers complanadines A, B, D, and E (**45–48**) from *D. complanatum* were reported to induce secretion of neurotropic factors from human astrocytoma cells; unfortunately the purity of the alkaloids tested was not stated [38,39]. Synthetic complanadine A (**45**) was shown to be a highly selective agonist on the pain-related MrgprX2 receptor expressed in neurons, while lycodine (**2**), which is one-half of the dimer, was inactive [40]. Again, the purity of the compound used was not mentioned. Alkaloid fractions from *L. clavatum* and *D. complanatum* have shown antiprotozoal activity together with the absence of cytotoxicity towards mammalian L6 cell lines [11], and *L. clavatum* and *D. thyoides* fractions have shown antioxidant effects and AChE inhibition *in vivo* in rats [14]. The active constituents were not determined. Inhibition of the enzyme AChE is by far the most studied activity for the lycopodium alkaloids, and the lycodane-type huperzine A (**Fig. 2**) is the most potent inhibitor found and is being studied as a possible drug lead against Alzheimer's disease [15,16,41]. In general, the lycodane-type alkaloids seem to be more potent AChE inhibitors than the lycopodane type [6,7,16,42] and lycopodine (**1**) itself is inactive [1].

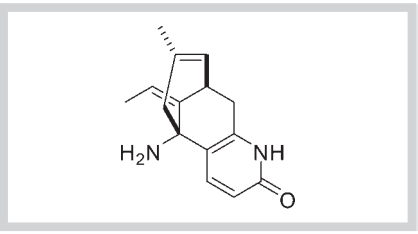


Fig. 2 Huperzine A.

Diphasiastrum and Lycopodium Alkaloids

Out of the 23 species of *Diphasiastrum* presented, the alkaloid content of 11 species has been studied to some extent. The results are summarized in **Table 2** and the alkaloids are grouped according to structural types. The chemical structures are shown in **Fig. 3** (lycopodine class), **Fig. 4** (lycodine class), and **Fig. 5** (fawcettimine class and unclassified) with a number for each structure. The trivial names of these alkaloids can be rather confusing and do not always indicate the structural relationship between compounds. In the following text, structures are sometimes referred to by numbers only.

The *Diphasiastrum* species produce alkaloids that exhibit a high degree of chemical diversity both with respect to carbon skeletons and substituent patterns. The widely distributed lycopodine (**1**) has been found in all of the investigated *Diphasiastrum* species, except in *Diphasiastrum fawcettii* (F.E. Lloyd & Underw.) Ho-

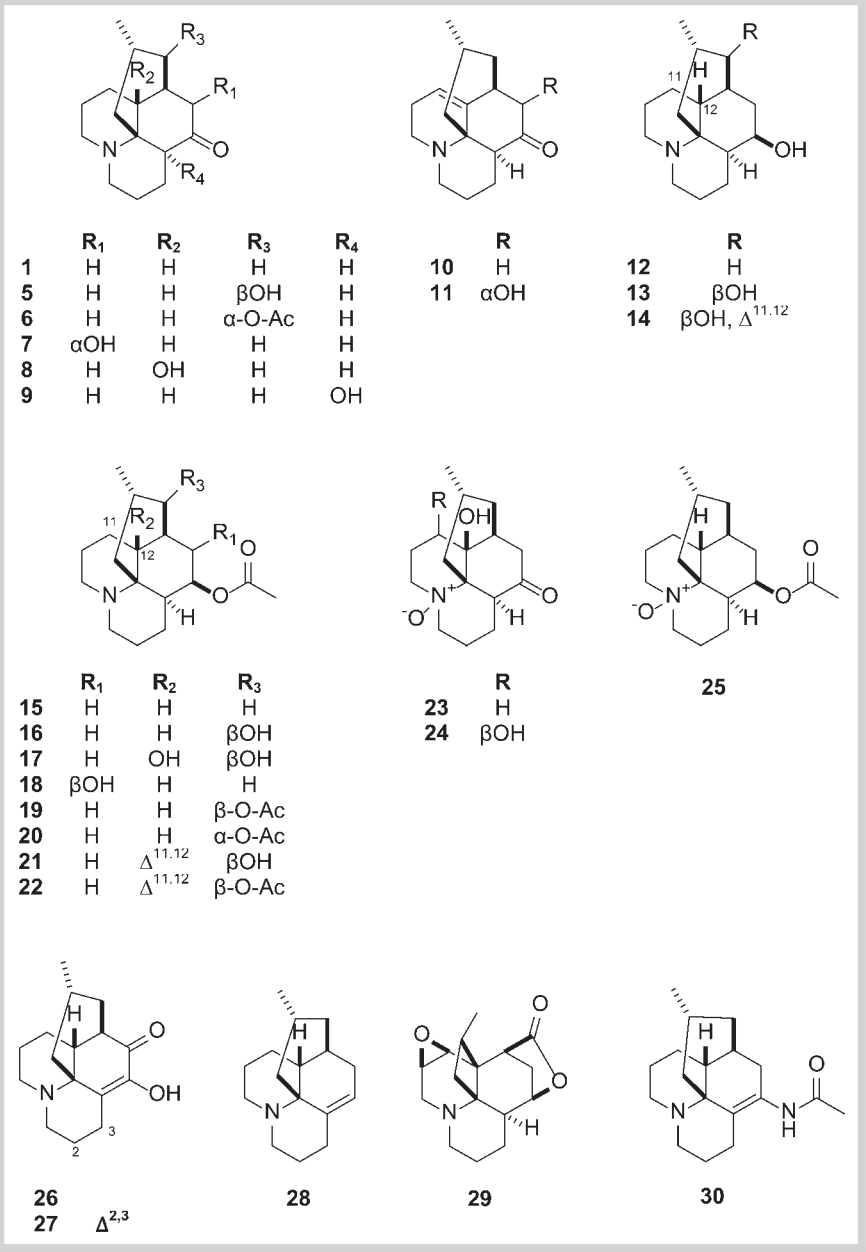
Table 2 Lycopodium alkaloids reported from *Diphasiastrum* species (February 2015). The species marked in bold are found in Europe.

Species	Alkaloids		
	lycopodane-type	**lycodane-type**	**fawcettimine-type**
D. alpinum	lycopodine (**1**) [1, 44], lycodoline (**8**) [1], anhydrolycodoline (**10**) [1], clavolonine (**5**) [1, 44], lycoclavine (**18**) [44], acetylfawcettiine(**19**) [1], acetylepiclavolonine (**6**) [1], acetyllofoline (**20**) [1]	des-N-methyl-α-obscurine (**37**) [44]	
D. carolinum	lycopodine (**1**) [47], lycodoline (**8**) [48], anhydrolycodoline (**10**) [48], dihydrolycopodine (**12**) [47]		
D. complanatum ssp. *complanatum* ssp. *montellii*	lycopodine (**1**) [38, 47, 49], complanadine C (**31**) [50], diphaladine A (**24**) [49], 6α-hydroxylycopodine (**7**) [49], lycopladine E (**25**) [51], lycoposerramine K (**11**) [49], obscurumine A (**23**) [49], 12-deoxyhuperzine O (**26**) [13]	lycodine (**2**) [49, 52, 53], des-N-methyl-α-obscurine (**37**) [49], des-N-methyl-β-obscurine (**40**) [49], complanadine A (**45**) [38, 52, 53], complanadine B (**46**) [38], complanadine D (**48**) [50], complanadine E (**47**) [54], 11-hydroxylycodine (**33**) [53], lyconadin D (**41**) [54], lyconadin E (**42**) [54], lycopladine F (**34**) [55], lycopladine G (**35**) [55], N-methyl-lycodine (**32**) [47]	lycoflexine (**54**) [49], lycopladine B (**49**) [56], lycopladine C (**50**) [56], lycopladine D (**51**) [56], phlegmariurine B (**53**) [49]
Unclassified alkaloids	lyconadin A (**57**) [53, 56], lyconadin B (**56**) [56], lyconadin C (**59**) [57], lyconadin F (**58**) [57], lycopladine A (**60**) [56, 58], lycopladine H (**61**) [59], lycospidine A (**62**) [13]		
D. digitatum	lycopodine (**1**) [60, 61], dihydrolycopodine (**12**) [60], acetyldihydrolycopodine (**15**) [62], clavolonine (**5**) [63], annotinine (**29**) [63], flabelliformine (**9**) [61], flabelline (**30**) [64]	lycodine (**2**) [63], des-N-methyl-α-obscurine (**37**) [63], α-obscurine (**36**) [65], β-obscurine (**39**) [65], flabellidine (**43**) [63], hydroxy-des-N-methyl-α-obscurine (**38**) [63]	
D. fawcettii	lycodoline (**8**) [66], acetylfawcettiine (**19**) [67], acetyllycofoline (**21**) [48], deacetylfawcettiine (**13**) [67], diacetyllycofoline (**22**) [67], fawcettiine (**16**) [66, 67], lycofawcine (**17**) [68, 69], lycofoline (**14**) [67]	lycodine (**2**) [68], des-N-methyl-α-obscurine (**37**) [68]	fawcettidine (**52**) [66], fawcettimine (**3**) [66, 70], lycopodium base R (**55**) [71]
D. henryanum	lycopodine* (**1**) [72], huperzine E* (**27**) [72], lycodoline* (**8**) [72]	lycodine* (**2**) [72], huperzinine* (**44**) [72]	
D. x *issleri*	lycopodine (**1**) [47]		
D. sabinifolium	lycopodine (**1**) [47]		
D. sitchense	lycopodine (**1**) [47], clavolonine (**5**) [47]	α-obscurine (**36**) [47]	
D. thyoides	lycopodine (**1**) [14, 47, 73], lycodoline* (**8**) [14], anhydrolycodoline* (**10**) [14], dihydrolycopodine (**12**) [48], clavolonine (**5**) [48], acetyldihydrolycopodine (**15**) [14, 47, 73], acetylfawcettiine (**19**) [47, 73], deacetylfawcettiine (**13**) [48], fawcettiine (**16**) [47, 73]	lycodine* (**2**) [14], α-obscurine* (**36**) [14], flabellidine (**43**) [14, 47]	
D. tristachyum	lycopodine (**1**) [47, 74], acetyldihydrolycopodine (**15**) [48], anhydrodihydrolycopodine (**28**) [47], dihydrolycopodine* (**12**) [74]	lycodine (**2**) [47, 74]	

* Indicates compounds identified by mass spectrometry only

lub. So far, lycopodine (**1**) alone is identified from *Diphasiastrum sabinifolium* (Willd.) Holub and *D.* x *issleri*, but it has also been described from *Diphasiastrum sitchense* (Rupr.) Holub along with clavolonine (**5**) and the lycodane-type α-obscurine (**36**). *D. fawcettii* produces two lycodane-type, **2** and **37**, three fawcettimine-type, **3, 52**, and **55**, and eight lycopodane-type alkaloids; unexpectedly, the widespread lycopodine (**1**) is not included. In *Diphasiastrum digitatum* (Dill. ex A. Braun) Holub, we have seven lycopodane-type and six lycodane-type alkaloids, as listed in **Table 2**, including lycodine (**2**), which is common amongst *Diphasiastrum* species, α- (**36**) and β-obscurine (**39**), and flabellidine (**43**). Flabellidine is also found in *D. thyoides* along with lycodine (**2**) and α-obscurine (**36**) and nine lycopodane-type alkaloids. *Diphasiastrum henryanum* (E. D. Br. & F. Br.) Holub collected in Tahiti, French Polynesia, was recently studied and five known alkaloids were identified by mass spectrometry. Two of these, huperzine E (**27**) and huperzinine (**44**), are rare and were reported in trace amounts [43]. They have not been described from other *Diphasiastrum* species and their existence in *D. henryanum* would need to be confirmed by other methods such as NMR spectroscopy. Huperzinine (**44**) in particular needs to be confirmed because it has a huperzine A-like structure with a free amino group, which would be new to *Diphasiastrum*.

The widely distributed heterogeneous *D. complanatum* is the most intensively studied species and several different structures are described. Both lycodane- and lycopodane-types are found (**Table 2**), as well as several dimers (**31, 45**–**48**) together with lyconadines A, B, C, and F (**56**–**59**), lycopladine A (**60**) and H (**61**), and lycospidine A (**62**) that do not belong to any of the established structural groups (grouped as unclassified) and have not been isolated from other *Diphasiastrum* or Lycopodiaceae species. Lycoflexine (**54**), an unusual fawcettimane-type alkaloid, is only found in *D. complanatum* so far. From the synonym list in **Table 1**, we can see that the name *L. complanatum* and *D. complanatum* has been used widely across the different species of this taxon, and it could be that some of the studies on the alkaloid of *D. complanatum* suffer from a lack of homogenously identified plant material due to the non-consistency in classification.



	R_1	R_2	R_3	R_4
1	H	H	H	H
5	H	H	βOH	H
6	H	H	α-O-Ac	H
7	αOH	H	H	H
8	H	OH	H	H
9	H	H	H	OH

	R
10	H
11	αOH

	R
12	H
13	βOH
14	βOH, $\Delta^{11.12}$

	R_1	R_2	R_3
15	H	H	H
16	H	H	βOH
17	H	OH	βOH
18	βOH	H	H
19	H	H	β-O-Ac
20	H	H	α-O-Ac
21	H	$\Delta^{11.12}$	βOH
22	H	$\Delta^{11.12}$	β-O-Ac

	R
23	H
24	βOH

Fig. 3 Lycopodane-type structures found in the *Diphasiastrum* genus.

Four out of six *Diphasiastrum* species that grow in Europe (shown in bold in ▶ **Table 1**) have been investigated. *D. complanatum, D. alpinum*, and *D. tristachyum* have been studied to some extent, and the hybrid *D.* x *issleri* (AC hybrid) has been shown to produce lycopodine (**1**), as do both parent species. The other two hybrids *D.* x *oellgaardii* (AT hybrid) and *D.* x *zeilleri* (CT hybrid) were not investigated. It would be interesting to know how the capacity to produce different types of lycopodium alkaloids enfolds in the hybrid plants compared to the parents; this would require careful authentication of the plant material used. *D. tristachyum* produces lycodine (**2**) and lycopodine (**1**) and three derivatives of lycopodine (**12, 15, 28**), while *D. alpinum* produces lycopodine (**1**), clavolonine (**5**), lycodoline (**8**), anhydrolycodoline (**10**), and some acetylated derivatives (**6, 18**–**20**), all of the lycopodane type. The first study on *D. alpinum* was on a European (Tyrol) collection [44] and reported des-N-methyl-α-obscurine (**37**) and lycoclavine (**18**), but this could not be confirmed by our recent study on the Icelandic *D. alpinum* [1]. In Iceland, *D. alpinum* is genetically isolated as it is the only *Diphasiastrum* species growing on the Mid-Atlantic Ridge far from the continents on each site. This, along with other environmental factors, could explain differences in the alkaloid patterns. Another thing that we noticed when studying *D. alpinum* [1] was that it contained a considerably lower total amount of alkaloids, i.e., 0.58 mg/g dry plant material, compared to 2.5 and 3.6 mg/g, respectively, for *Huperzia selago* (L.) Bernh. and *L. annotinum* previously investigated by our group [45, 46]. It is an open question if *Diphasiastrum* species in general have lower total alkaloid content than *Huperzia* and *Lycopodium* species.

General Discussion and Conclusion

The chemotaxonomical significance of the alkaloid pattern for the *Diphasiastrum* genus is difficult to comprehend on the basis of the present knowledge. This is not unexpected for a group of

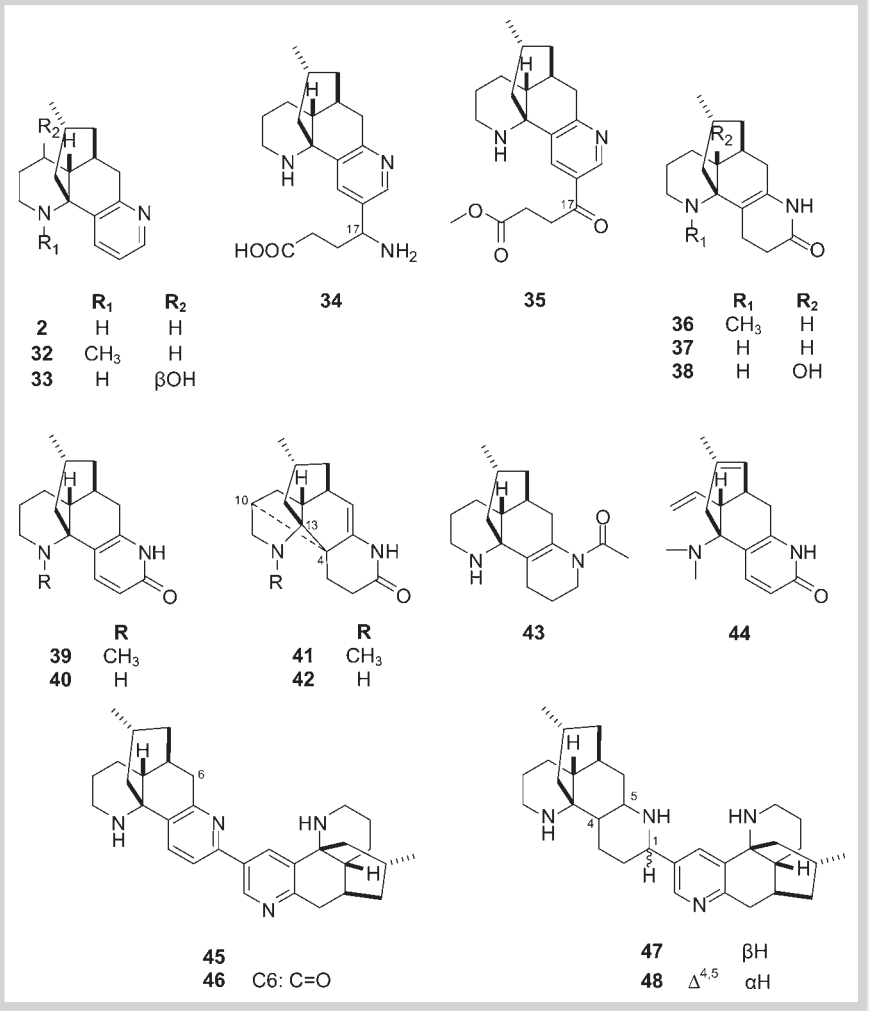


Fig. 4 Lycodane-type structures found in the *Diphasiastrum* genus.

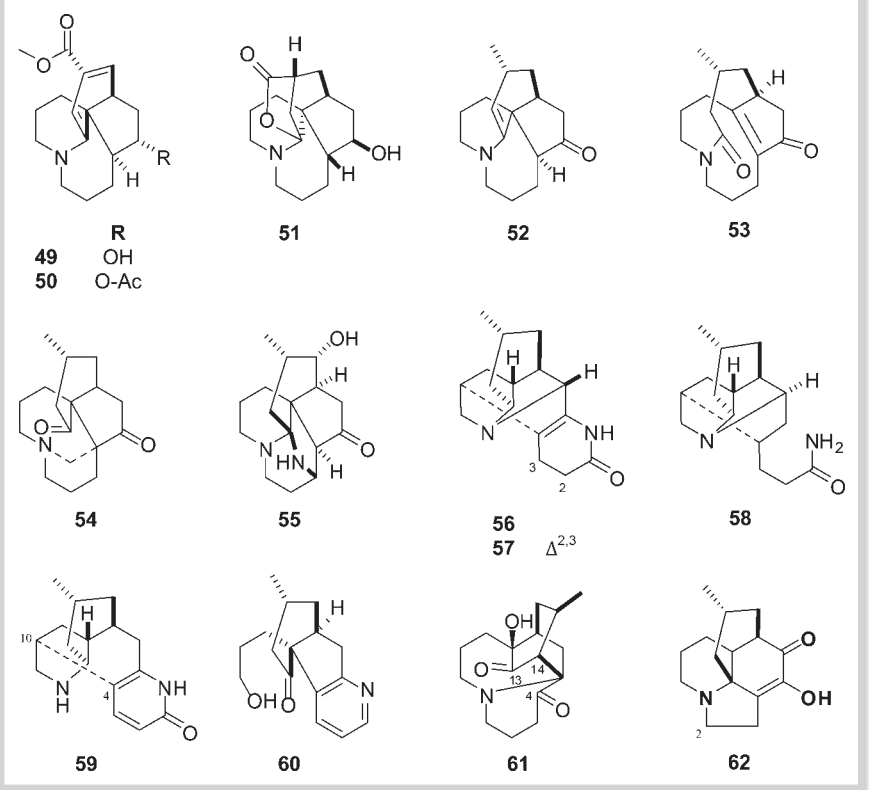


Fig. 5 Fawcettimane-type (**49**–**55**) and unclassified (**56**–**62**) structures found in plant species of the *Diphasiastrum* genus. Note that the structure of fawcettimine (**3**) is shown in ◉ **Fig. 1**.

plants where gene flow and hybridization of species is common. In addition, phytochemical studies of *Diphasiastrum* species might in some cases suffer from inaccurate identification of plant material used due to this complex taxonomical status [28], which again would influence the reported pattern of alkaloids across species. A standardized DNA barcoding method to assist with the taxonomic identification of *Diphasiastrum* plant material would certainly be appreciated for future studies in this area.

However, it can be concluded that lycopodane-type alkaloids are the most frequent structural type isolated from *Diphasiastrum*, which also applies to Lycopodiaceae in general, followed by the lycodane type. Fawcettimane-type alkaloids are found in two species and no alkaloids fall into the phlegmarine class. Most of the alkaloids found in *Diphasiastrum* are also found in other genera of Lycopodiaceae, although *D. complanatum* produces some unique structures such as, firstly, the dimers complanadine A–E and, secondly, a few newly discovered, unclassified structures, lycospidine (**62**), lycopladines A (**60**) and F (**61**), and lyconadines A–C and F (**56**–**59**), which have not been found elsewhere. Although these alkaloids could have taxonomical significance, it is too early to conclude if they are confined to this particular species, or to the *Diphasiastrum* genus. It is worth noting that the strong AChE inhibitor huperzine A is not found in any of the *Diphasiastrum* species and this lycopodium alkaloid seems to be restricted to the genus *Huperzia*. The most common lycodane-type alkaloids found in *Diphasiastrum* are lycodine (**2**), α-obscurine (**36**), and des-N-methyl-α-obscurine (**37**). To conclude, the present knowledge of the lycopodium alkaloids and their distribution in *Diphasiastrum* and *Lycopodium* species is not sufficient for chemotaxonomical distinction of the two genera.

Club mosses have been used in folk medicines as whole plants or extracts, and sometimes crude extracts are reported to have a given bioactivity. The compounds responsible might be lycopodium alkaloids or, alternatively, some other secondary metabolites in the extracts. The results of such experiments would need to be confirmed using pure compounds. *Diphasiastrum* species, e.g., *D. complanatum, D. alpinum*, and *D. thyoides*, have been used for medicinal purposes to treat conditions such as inflammation, infections, and neurological disorders. Like other club mosses, these species produce an array of lycopodium alkaloids that have mostly not been tested for bioactivity. However, studies have shown that complanadine A (**45**) has interesting neurological effects and the few studies that have been conducted on lycopodium alkaloids in general, including huperzine A, indicate that they can be expected to have low cytotoxicity towards mammalian cells and favorable pharmacological properties. Therefore, more candidates from this fascinating group of natural compounds could turn out to be interesting lead compounds for drug development. The club mosses, including the *Diphasiastrum* species, are slow-growing plants that are vulnerable to exploitation and therefore it is important to develop synthetic or other alternative methods to obtain the lycopodium alkaloids in sufficient quantities for future pharmacological studies.

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Conflict of Interest

The authors declare no conflict of interest.

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