

# Morphometric variation and ecological niche differentiation in *Hyophila apiculata* and *H. involuta* from karst microhabitats in Kanchanaburi, Thailand

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**Abstract.** Sawangproh W. 2024. Morphometric variation and ecological niche differentiation in *Hyophila apiculata* and *H. involuta* from karst microhabitats in Kanchanaburi, Thailand. *Biodiversitas* 25: 4551-4560. Karst environments, characterized by limestone and dolomite formations, host diverse ecosystems shaped by unique geological features such as sinkholes, caves, and underground rivers. These landscapes foster adaptations among flora and fauna, showcasing phenotypic plasticity that enables species to thrive in challenging conditions. This study examines the morphometric variation between *Hyophila apiculata* M.Fleisch. and *H. involuta* (Hook.) A.Jaeger in arid karst microhabitats of Kanchanaburi Province, Thailand. Surveys conducted across 86 sampling points revealed predominantly allopatric populations, with *H. apiculata* present in 26 sites (30%) and *H. involuta* in 58 sites (68%). Sympatric occurrences were rare, found at only two points (2%). Leaf morphometric analyses demonstrated that *H. involuta* exhibits wider leaves and larger leaf areas compared to *H. apiculata*, which features longer and wider median and apical leaf cells with thicker cell walls. Principal Component Analysis (PCA) indicated distinct morphological clusters for the two species, though some overlap was observed, suggesting the possibility of hybridization or transitional forms. These findings underscore the ecological niche differentiation and adaptive strategies of these moss species, enhancing our understanding of plant diversity and resilience in calcareous environments. This research contributes to the broader conservation efforts in these sensitive areas, emphasizing the need for preserving karst ecosystems that harbor unique biological communities and serve as indicators of environmental health. Such insights are critical for developing effective conservation strategies aimed at safeguarding the rich biodiversity found in karst landscapes.

**Keywords:** *Hyophila apiculata*, *Hyophila involuta*, microclimates, morphometric analysis, Western Thailand

## INTRODUCTION

Karst microhabitats, formed by the dissolution of carbonate rocks such as limestone and dolomite (Ford 2004), exhibit a variety of unique geological features, including sinkholes, caves, underground rivers, and jagged limestone formations (Waele and Gutiérrez 2022). These distinct characteristics create a wide range of environmental conditions, which in turn have prompted remarkable adaptations among both flora and fauna (Grismer et al. 2020). Wildlife in karst areas showcases impressive phenotypic plasticity, demonstrating their ability to adapt to the unique geological and ecological conditions of limestone landscapes (Li et al. 2022). The dynamic interplay of geological processes and ecological factors creates a mosaic of habitats that challenge the survival of organisms, necessitating diverse adaptive strategies (Zhu et al. 2017). For instance, amidst sinkholes, caves, and underground rivers, flora and fauna traverse a diverse array of habitats marked by fluctuating water levels (Bátori et al. 2023), variable nutrient availability (Chen et al. 2018), extreme darkness within caves (Engel 2007), and temperature gradients (Xie et al. 2021). Such variability demands a range of adaptive responses, from morphological adaptations to behavioral modifications (Dong et al. 2022; Zhao et al. 2024). These adaptive responses are key to

survival, as species must navigate the complex and often harsh conditions presented by the karst environment.

In karst regions, many plants exhibit xerophytic traits to cope with the often dry, nutrient-poor conditions. For example, plants commonly found in these areas may have reduced leaf size—a typical xerophytic adaptation aimed at minimizing water loss through transpiration (Zhang et al. 2021). They may also possess leathery, waxy, downy, or thick leaves, all of which help conserve moisture and protect against harsh environmental conditions (Martinson et al. 2019). Additionally, mesophyll differentiation is often more distinct in plants growing in these challenging habitats, further aiding their survival (Xiong et al. 2022). Similarly, fauna in karst environments, particularly cave-adapted species, have evolved unique traits to thrive in these specialized habitats. Troglodites, such as blind cavefish and various cave-dwelling invertebrates, display highly specialized sensory adaptations, allowing them to navigate and survive in the perpetual darkness of subterranean ecosystems (Leal-Zanchet and Marques 2018). This remarkable phenotypic plasticity enables both flora and fauna to exploit ecological opportunities while mitigating the threats posed by these dynamic landscapes. The interplay between genetic potential and environmental pressures is evident in the evolutionary trajectories observed across diverse taxa in karst ecosystems (Arroyave

and De La Cruz-Fernández 2021). Phenotypic plasticity, the ability of organisms to produce different phenotypes in response to environmental variation, is particularly crucial in these settings (Lafuente and Beldade 2019; de la Mata and Zas 2023).

Bryophytes, a group of non-vascular plants that include mosses, liverworts, and hornworts, are particularly noteworthy for their ability to adapt to a wide range of environmental conditions, including the often harsh and variable microhabitats of karst landscapes (Meng et al. 2023). Despite their relatively simple body structures, bryophytes display remarkable plasticity in response to environmental factors such as light, moisture, gravity, and nutrient availability (Mohanasundaram and Pandey 2022). This plasticity is manifested in their varied growth forms (Coe et al. 2024), physiological responses (Širka et al. 2019), leaf sizes (Buryová and Shaw 2005), and reproductive strategies (Nath and Bansal 2015), all of which are finely tuned to the specific conditions of their microhabitats. In karst environments, bryophytes frequently encounter high calcium carbonate content (Meng et al. 2023), limited water availability (Geekiyanage et al. 2019), and fluctuating temperatures (Ren et al. 2021). To thrive in these conditions, bryophytes have evolved a suite of adaptations, such as desiccation tolerance and efficient nutrient uptake, allowing them to successfully colonize these challenging limestone-rich landscapes (Yan et al. 2021). These adaptations not only underscore the ecological resilience of bryophytes but also provide valuable insights into the broader field of plant adaptation to extreme and calcareous environments.

The objective of this study is to examine the morphometric variation between *Hyophila apiculata* M.Fleisch. and *H. involuta* (Hook.) A.Jaeger populations inhabiting arid karst microenvironments in Kanchanaburi Province, Thailand. *H. apiculata* typically grows on rocks and soil, primarily at altitudes below 1000 meters, while *H. involuta* is found in more humid environments, such as stream banks on silty and rocky substrates, often at altitudes below 2500 meters. Although these species occupy overlapping habitats, they display distinct ecological preferences. The author anticipates that investigating the morphometric adaptations of these moss species will offer valuable insights into their ecological roles within karst ecosystems. By understanding their specific adaptations, we can better appreciate how these mosses influence local microclimates, contribute to soil stability, and support overall biodiversity within these unique environments.

## MATERIALS AND METHODS

### Study area

Kanchanaburi Province, situated in Western Thailand and bordering Burma (Myanmar), lies approximately 129 kilometers west of Bangkok. Geographically, it extends between 13.45-15.40 degrees north latitude and 98.15-99.53 degrees east longitude. The province's northern and

western regions are characterized by rugged mountains and plateaus, forming part of the Thanon Thong Chai Mountain range. These areas are enveloped in lush forests, which add to the province's rich ecological diversity. The vegetation in Kanchanaburi includes bamboo, dry evergreen, and mixed deciduous forests, creating a mosaic of habitats that support an array of flora and fauna. Additionally, significant river systems, particularly the Kwai Noi and Kwai Yai Rivers, flow through the eastern and southern parts of the province. These rivers form extensive floodplains, which are vital for the region's agriculture.

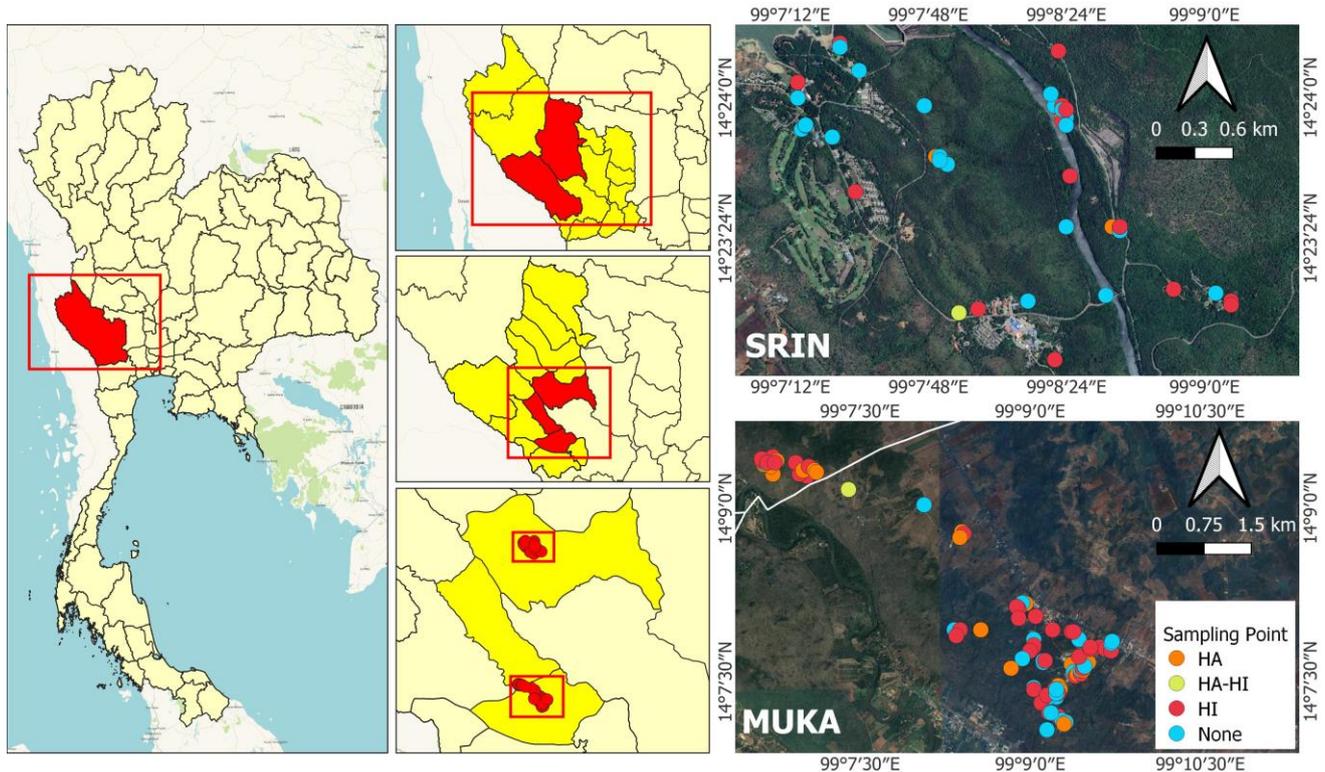
The province experiences three distinct seasons: a cool season from November to February, a hot season from March to May, and a rainy season from June to October (Realistic Asia 2024). With an elevation ranging between 100 and 900 meters above sea level (masl), Kanchanaburi has an average annual temperature of 27.5°C. The highest recorded temperature is 39.1°C, observed in April, while the lowest, 14.6°C, was recorded in December (Marod et al. 2010). These climatic variations, along with the province's topographical and vegetative diversity, contribute to a unique environment that supports a wide variety of plant species, including *Hyophila* mosses.

This study focused on surveying *Hyophila* species in two distinct mixed deciduous forest locations: the Mahidol University Kanchanaburi Campus (MUKA) and the Srinakarind Dam (SRIN), both situated within Kanchanaburi (Figure 1). Given their diverse microclimates, elevation ranges, and proximity to river systems, these sites provide ideal conditions for investigating the ecological and morphometric variations of *Hyophila* species.

(i) Mahidol University Kanchanaburi Campus (MUKA) is in Sai Yok District and spans an area of approximately 8 square kilometers, with elevations ranging from 130 to 290 masl. Previous research carried out during the 2012-2014 Plant Genetic Conservation Project under Her Royal Highness Princess Maha Chakri Sirindhorn has cataloged various flora and fauna in the area, including ginger plants, orchids, *Vigna* beans, land mollusks, soil arthropods, butterflies, small mammals (such as bats), and medicinal plants. However, no comprehensive survey of bryophyte species has been conducted in this region.

(ii) The Srinakarind Dam (SRIN) area, also part of the Plant Genetic Conservation Project under Her Royal Highness Princess Maha Chakri Sirindhorn, is located at elevations between 70 and 210 masl. Since 2009, biodiversity surveys by Mahidol University researchers have cataloged perennial plants, flowering plants, medically significant arthropods, soil microarthropods, animals, essential oil-producing plants, land and freshwater mollusks, and medicinal mushrooms. Despite these extensive studies, the diversity of bryophytes in this area remains unexplored.

These two sites offer an opportunity to deepen our understanding of bryophyte diversity, particularly their adaptation to the microhabitats of Kanchanaburi's mixed deciduous forests.



**Figure 1.** Geographic locations of sampling points for *Hyophila apiculata* and *H. involuta* in two karst areas of Kanchanaburi Province, Thailand: MUKA, representing Mahidol University Kanchanaburi Campus, and SRIN, representing the Srinakarind Dam. Note: HA: Allopatric populations of *H. apiculata* (n = 26); HI: Allopatric populations of *H. involuta* (n = 58); None: No populations of *Hyophila* species observed (n = 46); HA-HI: Sympatric populations of *Hyophila* species (n = 2)

### Bryophyte sampling

Bryophyte surveys and sample collection were conducted in the MUKA and SRIN areas between July 2020 and April 2021. Field surveys were carried out daily between 9:00 a.m. and 4:00 p.m. to maximize the chances of observing and collecting bryophytes under optimal daylight conditions. During the study period, environmental data were recorded, indicating a mean air temperature of 25.36°C, with temperatures ranging from a low of 12.30°C to a high of 40.90°C. The mean relative humidity was 79.65%, with humidity levels fluctuating between 8.40% and 100.00%, as recorded by the Elitech® RC-51H Datalogger.

Bryophyte samples were collected using a random sampling method, in which a 50 cm × 50 cm square quadrat was systematically placed across various locations, following the sampling methodology outlined by Vanderpoorten et al. (2010). This quadrat approach ensured that samples were representative of the area's bryophyte diversity. Each time bryophytes were encountered; detailed observations were made regarding the surrounding environment and the type of substrate on which the bryophytes were growing. Substrates were classified based on the microhabitat criteria established by Vanderpoorten et al. (2010), which included categories such as terricolous (ground-dwelling), saxicolous (rock-dwelling), and others, depending on where the bryophytes were found.

Collected bryophyte samples were placed in 10 × 15 cm paper envelopes, ensuring that they were carefully handled to avoid damage. These envelopes served as voucher

specimens for scientific name verification, which were subsequently preserved at the Plant Herbarium of the Western Region, located at Mahidol University's Kanchanaburi Campus. Species-level identification of the collected bryophytes was carried out using dichotomous keys, specifically those developed by Eddy (1991) and Ajintaiyasil (2017). This classification process allowed for accurate taxonomic identification of the bryophyte species present in the surveyed areas, contributing valuable data to the study of bryophyte diversity in Kanchanaburi Province.

### Study species

*Hyophila apiculata* is characterized by its yellowish-green plants, which form turfs measuring between 3.0 and 5.0 mm in length. The stems are erect and simple, with leaves that curl inward when dry but spread out when moist, demonstrating an adaptation to fluctuating moisture conditions. The leaves are obovate oblong to spatulate in shape, with entire, apiculate margins, and specific cell structures that further distinguish the species. This moss is dioicous, meaning that male and female reproductive organs are found on separate plants. *H. apiculata* reproduces sexually, producing erect and cylindrical capsules that contain small, spherical, smooth spores. Its ecological preference is for shaded soil environments under trees. Geographically, *H. apiculata* is widely distributed across Thailand, especially in the northern regions, and can also be found in Australia, Brazil, Indonesia, Malaysia, and Thailand.

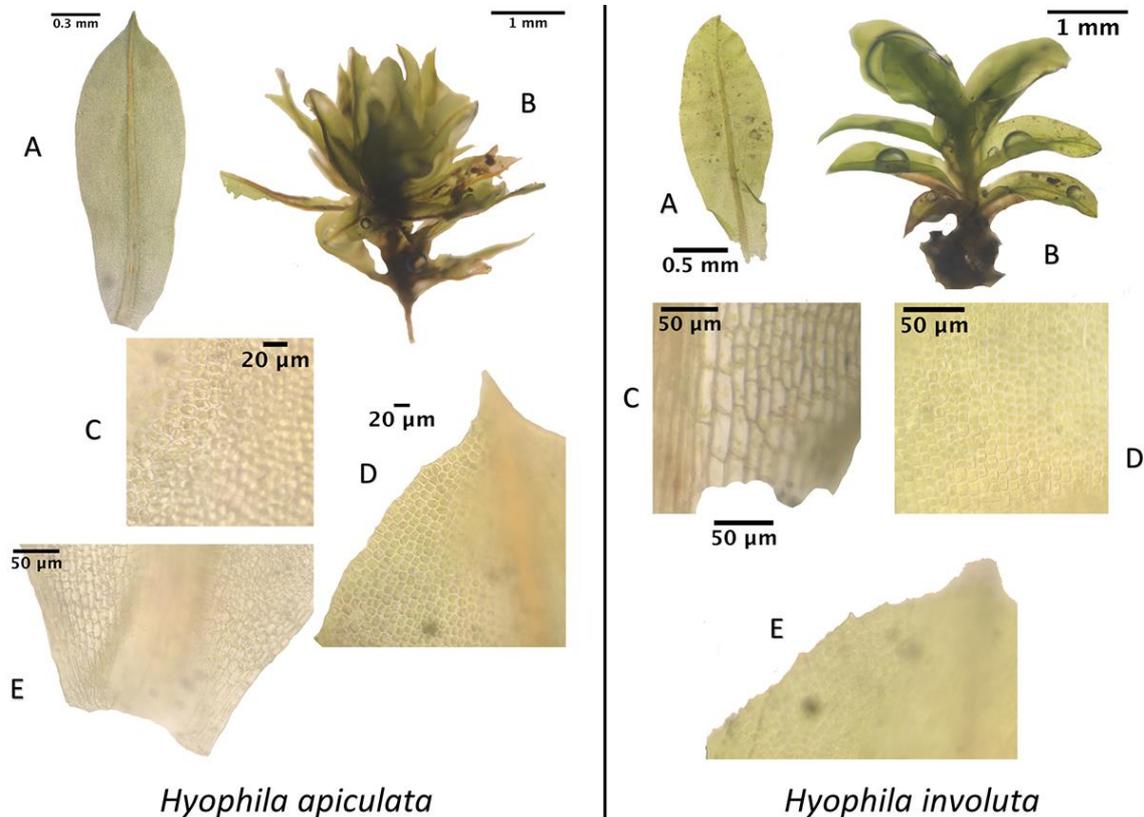
*Hyophila involuta* has several distinct characteristics that set it apart from *H. apiculata*. This species is also yellowish-green and forms dense turfs ranging from 3.0 to 6.0 mm in length. The erect stems bear leaves that are lingulate to spatulate in shape, curling inward when dry and spreading outward when moist, like *H. apiculata*. However, the leaves of *H. involuta* have serrated margins and apices that range from broadly acute to obtuse, while the upper leaf cells are small and thick-walled. Like *H. apiculata*, *H. involuta* is also dioicous. It reproduces both sexually and asexually. Sexual reproduction involves the production of erect and cylindrical capsules with spherical,

smooth spores, whereas asexual reproduction occurs through the formation of oval, yellowish-green gemmae. *H. involuta* is distributed globally and found in regions including Australia, Brazil, China, India, Japan, Thailand, and the United States. Ecologically, it thrives in sunnier habitats, growing on soil or rocks.

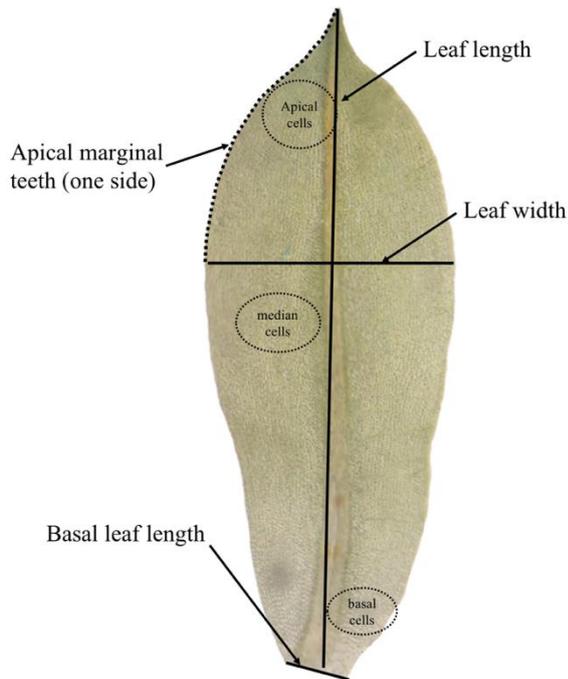
Further morphological and ecological differences between *H. apiculata* and *H. involuta* are illustrated in Figure 2 and summarized in Table 1, highlighting variations in leaf morphology, reproductive strategies, and substrate preferences.

**Table 1.** Comparison of morphological and ecological characters of two *Hyophila* species (Eddy 1991; Ajintaiyasil 2017)

Character	<i>H. apiculata</i>	<i>H. involuta</i>
Gametophyte plant	Short turfs measuring 3.0-5.0 mm	Short turfs measuring 3.0 to 6.0 mm
Leaf	Obovate-oblong to spatulate	Lingulate to spatulate
Leaf margin	Entire	Usually remotely and irregularly dentate at upper margin
Leaf apex	Apiculate	Broadly acute to obtuse, usually with a distinct mucro
Leaf costa	N/A	Strong, brownish or reddish, ending in the leaf tip
Leaf cell	Cells with firm walls	Quadrate at upper lamina cells, with firm but rarely strongly thickened walls
Basal leaf cell	Rectangular hyaline cells	Rectangular hyaline cells with thin walls
Capsule	Cylindrical	Frequently present, cylindrical, urn-shaped, 2-3 mm long
Seta	Up to 1 cm long, smooth, yellowish above, reddish at base	Up to 1.5 cm long, smooth, red-brown at the base, pale above
Microhabitat	On rocks and soil, mainly below 1000 m	On humid silt and rocks on stream banks below 2500 m



**Figure 2.** The comparison of plant habit, leaf structure, and leaf cells between *Hyophila apiculata* (left) and *H. involuta* (right) is as follows: A. Gametophyte leaf; B. Plant habit; C. Cells at the median part of the leaf; D. Cells at the leaf apex; E. Cells at the leaf base



**Figure 3.** Some selected leaf measurements are described in this research. All measurement variables are presented in Table 2

### Leaf morphometric study

From each sampling point, a single gametophyte shoot of each moss species was randomly selected for comprehensive morphological analysis. Approximately 10 leaves were delicately removed from about one-third of the length of the shoot tip using fine forceps and mounted on glass slides for microscopic examination. A complete, representative leaf from the sample was selected for photography under a compound microscope. Whole leaf structures were captured at 40× magnification, while leaf cells in the basal, median, and apical regions were photographed at 100× magnification. These images were captured using a digital camera attached to the microscope, enabling high-resolution documentation of the leaf morphology.

In this study, a total of 14 morphological traits, along with 4 derived characteristics, were measured for branch leaves (see Figure 3 for details). In total, 88 samples were analyzed, comprising 28 samples of *H. apiculata* and 60 samples of *H. involuta*. Leaf dimensions and specific characteristics were measured using the "Measure" tool in the ImageJ software, which provides precise image analysis. ImageJ, a widely recognized platform for scientific image processing, was developed by Wayne Rasband at the National Institutes of Health (NIH) (Schindelin et al. 2015). The tool allowed for a highly accurate assessment of the leaf traits critical for understanding the morphological differences between the two moss species.

### Statistical analyses

The 14 morphological leaf characters were first evaluated for normality using the Shapiro-Wilk test. Based on the outcome of the normality test, means were compared between *H. apiculata* and *H. involuta* using either an independent sample Student's t-test for normally

distributed data or the unpaired two-sample Wilcoxon test for data that did not follow a normal distribution. The non-parametric Wilcoxon test was employed in these cases to provide a robust comparison. The statistical analyses and visualizations were conducted using the *ggplot2* package within RStudio version 3.3.1 (RStudio Team 2016).

For species classification, all samples from both study areas - Mahidol University Kanchanaburi Campus (MUKA) and Srinakarind Dam (SRIN) - were pooled for each species based on descriptions by Eddy (1991) and Ajintaiyasil (2017). This combined dataset allowed for a comprehensive analysis of species-level differences.

To further explore patterns of variation in leaf morphological traits, Principal Component Analysis (PCA) was performed on the 14 measured leaf characters. The analysis was carried out using the R packages *FactoMineR* and *Factoextra*, which enabled a visualization of the major axes of variation between the two species. PCA provided insights into the key traits that distinguish the species and contributed to understanding their morphological diversity across the study areas.

## RESULTS AND DISCUSSION

### Distribution patterns of *Hyophila* species across sampling points

From a total of 86 sampling points, the researcher found that the majority of populations were allopatric, with a single *Hyophila* species present at each location. Specifically, *H. apiculata* was observed in 26 allopatric populations, accounting for 30% of the sampling points, while *H. involuta* was found in 58 allopatric populations, representing 68%. Sympatric populations, where both species coexisted at the same sampling location, were rare, with only two such instances (2% of the total). One instance occurred at Mahidol University Kanchanaburi Campus (MUKA), and the other at Srinakarind Dam (SRIN). These occurrences are depicted in Figure 1 and Table S1.

### Association between moss species and leaf shape

The distribution of leaf shapes in two moss species, *H. apiculata* and *H. involuta*, is depicted in Figure 4. Leaf shapes within these two species exhibit notable variability, highlighting the morphological diversity present within the genus. In *H. apiculata*, the predominant leaf shapes are elliptic (12 out of 28 leaves, or 43%) and obovate (9 out of 28 leaves, or 32%), suggesting a potential adaptive advantage in specific ecological niches. Conversely, *H. involuta* displays a different pattern, with the majority of leaf shapes being elliptic (26 out of 60 leaves; 43%) and oblong (11 out of 60 leaves, or 18%). This variance in leaf morphology may reflect differences in environmental adaptations or growth conditions between the two species. However, it is important to note that the association test conducted to evaluate the relationship between moss species and leaf shape yielded statistically insignificant results (Chi-square test: Chi-square value = 10.02, df = 5, p = 0.074).

### Association between moss species and microhabitat

Figure 5 indicates that the frequency of *H. apiculata* and *H. involuta* is notably higher on rocks (saxicolous habitats) than on soil (terricolous habitats). However, despite this observed trend, statistical analysis reveals no significant association between moss species and their microhabitats (Chi-square test: Chi-square value = 0.0029, df = 1,  $p = 0.957$ ).

### Leaf morphometric analysis

The results revealed notable differences in most of the leaf characters examined between *H. apiculata* and *H. involuta*. For instance, *H. involuta* exhibited significantly wider leaves and a larger leaf area compared to *H. apiculata*, with an apparently dentate margin (Figure 6). Conversely, *H. apiculata* displayed longer and wider median and apical leaf cells with thicker cell walls than *H. involuta* (Figure 6). However, both moss species showed no variation in basal leaf length, leaf length, basal cell length, basal cell width, and basal cell wall thickness (Figure 6).

### Multivariate analysis

The PCA analysis based on the leaf morphology dataset indicates that 51.41% of the observed variation is accounted for by the first three factors (PC1 = 22.71%, primarily summarizing variation in general leaf size, number of teeth at the apical leaf margin, and traits associated with basal leaf cells; PC2 = 18.25%, mainly reflecting leaf size and traits of median and apical leaf cells; PC3 = 10.45%, largely determined by traits of median and apical leaf cells; see Table 2). Plotting individual component scores along the first principal component (x-axis) and the second principal component (y-axis) (see Figure 7) revealed incomplete separation of *H. apiculata* and *H. involuta* species, with some individuals from each species partially overlapping the two clusters, predominantly filling the intermediate space between the two species.

### Discussion

The observed distribution of *Hyophila* species across the 86 sampling points provides insight into their ecological preferences and spatial overlap within the study area. The results indicate a predominance of allopatric populations, where each species tends to occupy distinct geographic locations without overlapping extensively with the other. Specifically, *H. apiculata* was found in 26 allopatric populations, constituting approximately 30% of the total sampling points, while *H. involuta* was more widespread, occurring in 58 allopatric populations, accounting for around 68% of the total. In contrast, sympatric populations, where both species co-occurred, were relatively rare, observed at only two sampling points (2% of the total). These points were identified at MUKA and SRIN, as illustrated in Figure 1 and detailed in Table S1. This spatial

pattern suggests that while *H. apiculata* and *H. involuta* can share habitats under certain conditions, they predominantly occupy separate ecological niches within the study area. The rarity of sympatric occurrences implies potential ecological differentiation or competitive exclusion mechanisms between the two species (Scriven et al. 2016). Understanding these patterns is crucial for assessing biodiversity dynamics and conservation strategies in karst ecosystems, where even subtle differences in species distributions can reflect significant ecological processes.

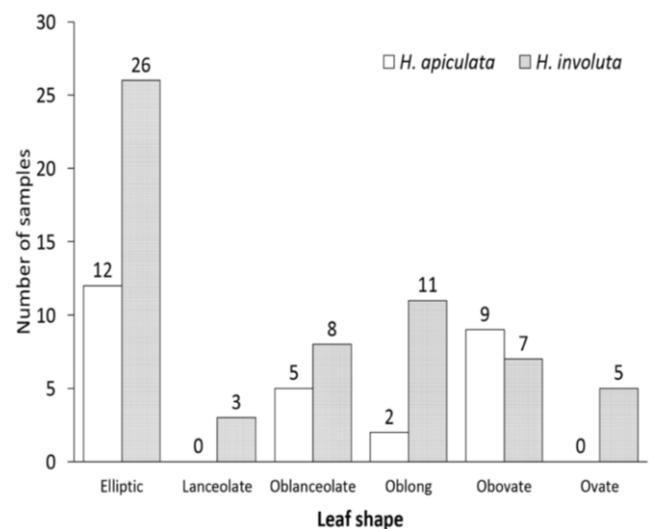


Figure 4. The frequencies of different leaf shapes for *Hyophila apiculata* and *H. involuta*

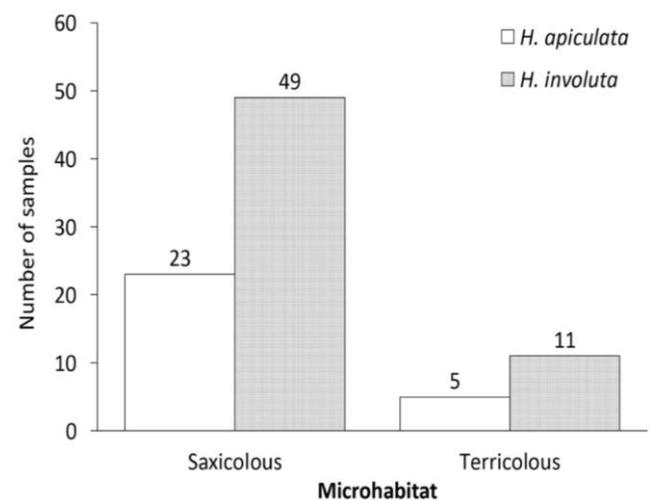
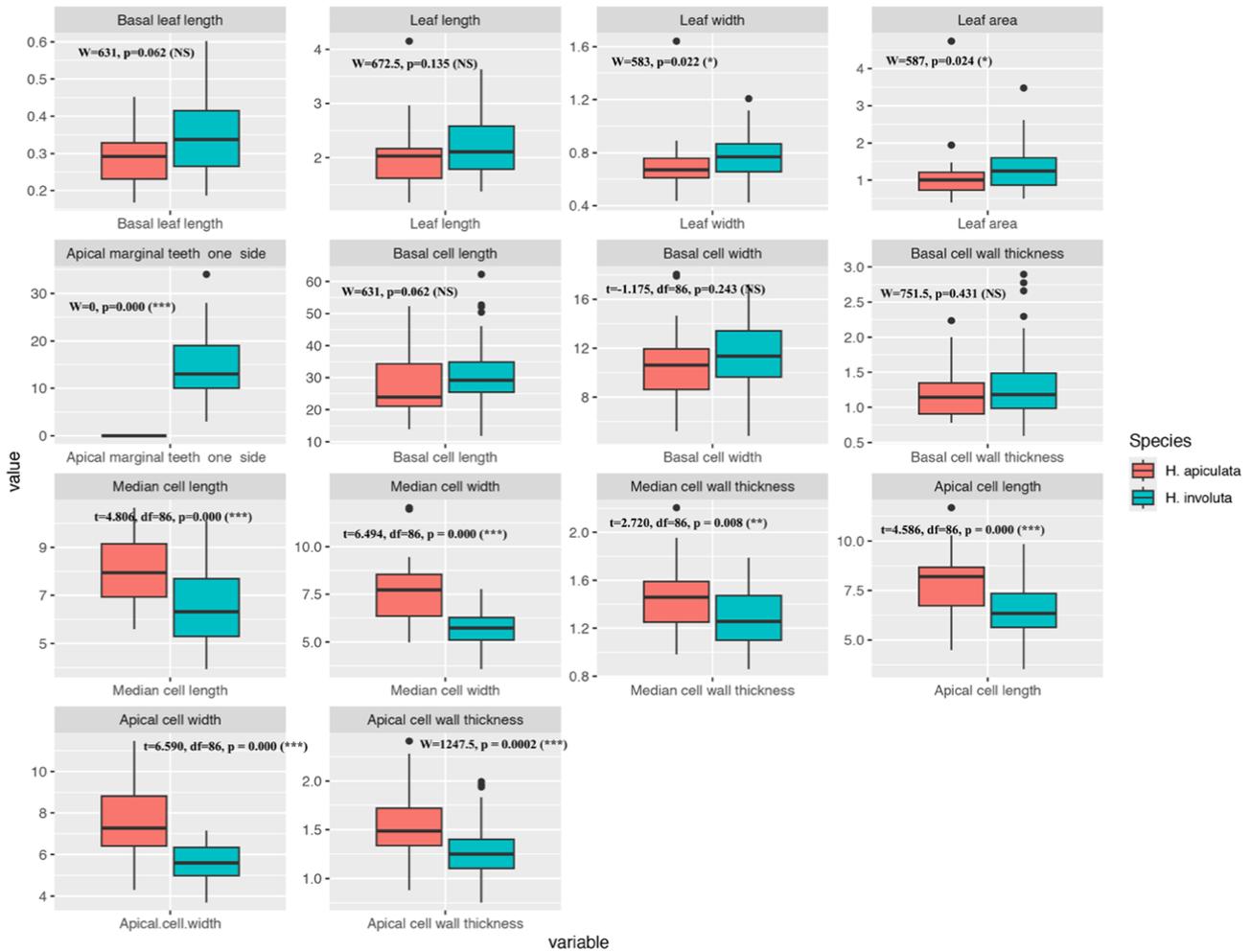


Figure 5. The frequencies of microhabitats of *Hyophila apiculata* and *H. involuta*

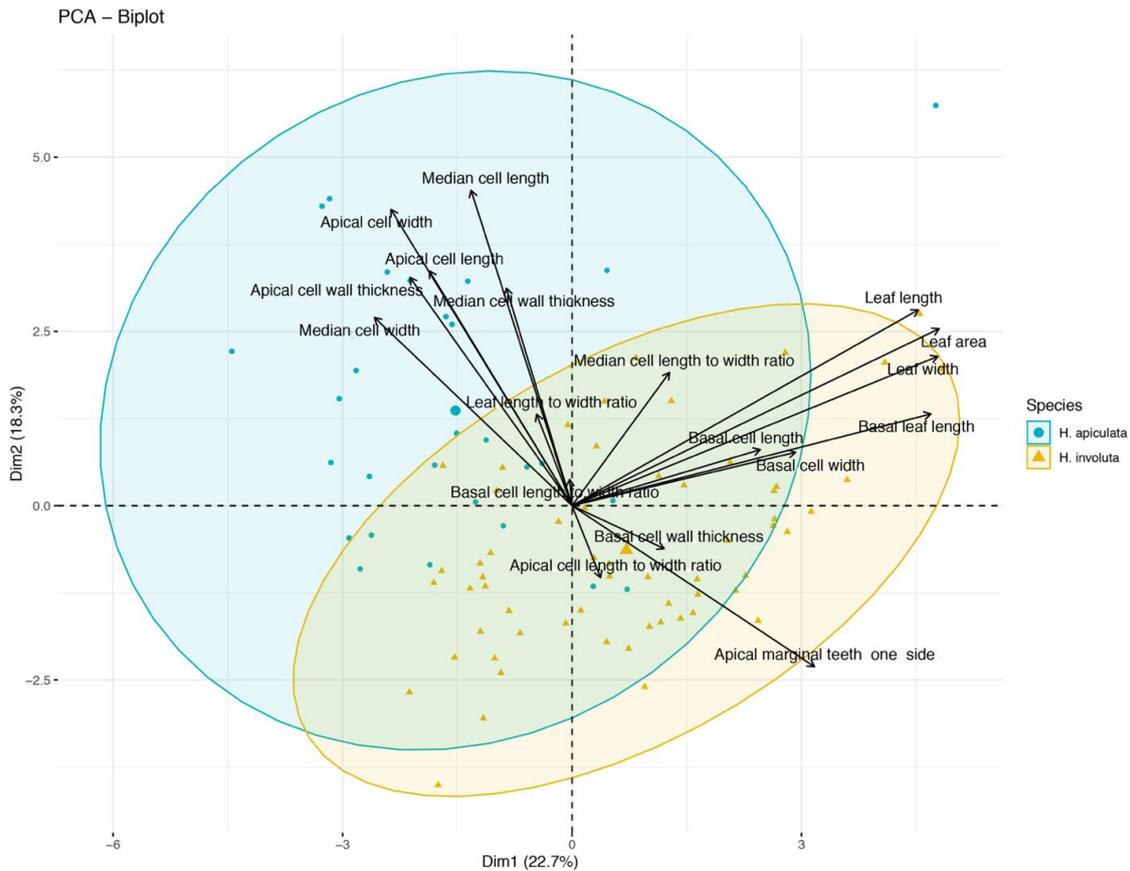


**Figure 6.** Box plots displaying leaf character measurements for *Hyophila apiculata* and *H. involuta*. Note: NS:  $p > 0.05$ , \*:  $p \leq 0.05$ , \*\*:  $p \leq 0.01$ , \*\*\*:  $p \leq 0.001$

Despite the observed differences in leaf shapes between the two moss species (Figure 4), the statistical analysis conducted to determine the association between moss species and leaf shape yielded insignificant results in this study. This implies that while there are distinct patterns in leaf shape distribution within each species, these patterns do not appear to be strongly linked to the species themselves. These findings prompt further exploration into the factors influencing leaf shape variation within two *Hyophila* species. Possible avenues of investigation could include environmental factors, genetic influences, or interactions with other biotic and abiotic elements in the mosses' habitats. Additionally, considering the intricacies of moss ecology and evolutionary dynamics, future studies might delve deeper into the functional significance of leaf shape variation within and across species, shedding light on the ecological and evolutionary processes shaping moss diversity and distribution.

In this study, the researcher observed both *Hyophila* species more frequently growing allopatrically (Figure 1) on rock and soil. This observation contradicts the findings of Ajintaiyasil (2017), which described *H. apiculata* as a terricolous species restricted to soil in deciduous dipterocarp forests at lower altitudes (approximately 285

masl). In contrast, *H. involuta* was classified as both a terricolous and saxicolous species in deciduous dipterocarp forests, lower montane rainforests, and lower montane coniferous forests at higher altitudes (294-1,179 masl). Despite the observed preference for saxicolous habitats, the statistical analysis aimed at discerning the association between moss species and microhabitats yielded non-significant results (Figure 5). These findings suggest that while there may be a preference for saxicolous habitats within the studied moss species, this preference does not translate into a significant association between moss species and microhabitat type. Several factors could contribute to this lack of association, including the presence of other ecological variables influencing habitat selection, such as moisture levels, light exposure, or substrate composition. Furthermore, it is essential to consider the potential limitations of the study, such as the spatial scale of sampling or the specific environmental conditions of the study site. Additionally, future research endeavors could delve deeper into understanding the ecological drivers behind microhabitat preferences in these moss species, potentially incorporating multifaceted approaches that encompass both field observations and experimental manipulations.



**Figure 7.** Principal component analysis was performed on 14 leaf characters using 88 specimens representing species of *Hyophila apiculata* and *H. involuta*. The first two axes (PC1 and PC2), accounting for a total of 40.96% of the variation, are displayed. Data points are colored and shaped according to the assigned species of the specimens. Encircled areas represent the 95% confidence level of the sample means, with the center of each cluster marked by a larger symbol. Factor loadings are presented in Table 2

**Table 2.** Factor loadings for the first three principal axes of a PCA based on leaf morphometrics of *Hyophila apiculata* and *H. involuta*. High scores (loadings>0.2) are bolded to denote variables contributing significantly to the variation explained by each principal component

Character	Factor loading		
	PC1	PC2	PC3
Basal leaf length (mm)	<b>0.398</b>	0.124	-0.004
Leaf length (mm)	<b>0.384</b>	<b>0.266</b>	-0.037
Leaf width (mm)	<b>0.405</b>	<b>0.203</b>	-0.157
Leaf length/ width ratio	-0.039	0.124	<b>0.210</b>
Leaf area (mm <sup>2</sup> )	<b>0.407</b>	<b>0.240</b>	-0.104
Apical marginal teeth (one-sided)	<b>0.269</b>	<b>-0.218</b>	0.095
Basal leaf cell length (µm)	<b>0.209</b>	0.076	-0.067
Basal leaf cell width (µm)	<b>0.248</b>	0.072	0.110
Basal leaf cell length/width ratio	-0.003	0.036	-0.189
Basal leaf cell wall thickness (µm)	0.102	-0.058	0.194
Median leaf cell length (µm)	-0.112	<b>0.428</b>	0.037
Median leaf cell width (µm)	<b>-0.219</b>	<b>0.255</b>	<b>-0.345</b>
Median leaf cell length/width	0.108	0.181	<b>0.293</b>
Median leaf cell wall thickness (µm)	-0.073	<b>0.295</b>	0.126
Apical leaf cell length (µm)	-0.158	<b>0.319</b>	<b>-0.409</b>
Apical leaf cell width (µm)	<b>-0.2001</b>	<b>0.402</b>	<b>0.218</b>
Apical leaf cell length/width	0.032	-0.098	<b>-0.604</b>
Apical leaf cell wall thickness (µm)	-0.180	<b>0.310</b>	0.137
Eigenvalue	4.087	3.286	1.880
Cumulative variance (%)	22.71	40.96	51.41

The comparison between *H. apiculata* and *H. involuta* revealed distinct differences in several leaf characteristics, highlighting the unique morphological features of each species. Specifically, significant variations were observed in leaf width and leaf area, with *H. involuta* demonstrating wider leaves and a larger overall leaf area compared to *H. apiculata*. Additionally, *H. involuta* exhibited a dentate margin, indicating further divergence in leaf morphology between the two species (Figure 6). These findings are consistent with Ajintaiyasil (2017), who observed larger dimensions of leaves for *H. involuta* than for *H. apiculata*. In contrast, *H. apiculata* displayed distinct features such as longer and wider median and apical leaf cells, accompanied by thicker cell walls compared to *H. involuta*. These differences in cell dimensions suggest potential adaptations to specific environmental conditions or ecological niches occupied by each species. The wider leaves and larger overall leaf area of *H. involuta* may indicate an adaptation to thrive in more humid microhabitats, while the longer and wider median and apical leaf cells with thicker cell walls in *H. apiculata* may reflect adaptations to drier microhabitats, as suggested by Eddy (1991).

Due to the study sites being in dry deciduous forests where wildfires often occur, ephemeral turf mosses like *H. apiculata* and *H. involuta*, which rapidly grow and develop, might be suitably adapted (Calabria et al. 2016). While

there is limited documentation on morphological adaptations in bryophytes, Proctor et al. (2007) and Stark et al. (2007) proposed that the development of reduced or minute gametophytic forms lacking branching could serve as a significant strategy to cope with dry conditions. This explanation can also be applied to our study species, such as the acrocarpous mosses *H. apiculata* and *H. involuta*, which typically exhibit small, unbranched growth forms and erect turfs in the arid karst areas of Kanchanaburi Province. Printarakul and Jampeetong (2020) observed two distinct forms of *H. involuta* based on their microhabitats, namely humid and arid areas. According to their study, *H. involuta* from wet habitats displayed larger gametophytic characteristics, such as stem height, leaf size, stem diameter, innermost perichaetia, and archegonia, compared to those from dry habitats.

Interestingly, despite the notable contrasts in certain leaf characteristics, both *H. apiculata* and *H. involuta* exhibited uniformity in several other traits, including basal leaf length, leaf length, basal cell length, basal cell width, and basal cell wall thickness (Figure 6). This consistency suggests a level of morphological stability or functional constraints governing these particular leaf attributes within the studied populations. These findings shed light on the intricate morphological diversity within two *Hyophila* species and highlight the importance of considering multiple leaf characters to discern species-specific traits accurately. The observed differences in leaf morphology between *H. apiculata* and *H. involuta* likely reflect adaptations to their respective habitats, microenvironments, or ecological niches, demonstrating the role of natural selection in shaping plant morphology. Furthermore, these results provide valuable insights into the taxonomic classification and ecological niche differentiation of moss species. Future studies could explore the genetic underpinnings of these morphological variations and investigate their ecological implications, contributing to a deeper understanding of plant adaptation and diversity in diverse ecosystems.

The partial overlap observed in the PCA plot (Figure 7) suggests that while there are discernible differences in leaf morphology between *H. apiculata* and *H. involuta*, there is also considerable variability within each species. This variability may stem from intraspecific variation, environmental influences, or genetic factors that contribute to the complexity of morphological differentiation within moss populations. Furthermore, the intermediate positioning of some individuals between the two species clusters implies the existence of transitional forms or hybridization events (Sawangproh et al. 2020a,b), indicating potential gene flow or shared ancestry between *H. apiculata* and *H. involuta*. These findings underscore the dynamic nature of species boundaries and highlight the importance of considering intraspecific variation and evolutionary processes in understanding plant diversity and speciation. Overall, the PCA analysis provides a comprehensive framework for elucidating patterns of morphological variation and highlighting the complexities inherent in species differentiation within moss populations. Further investigations incorporating genetic analyses and

ecological data could provide additional insights into the underlying mechanisms driving morphological divergence and species evolution in mosses.

To conclude, despite the variability in leaf shapes within the two *Hyophila* species, the statistical analyses revealed no significant associations between species and leaf shape distribution or microhabitats. This suggests that while certain morphological traits are distinct within each species, they do not strongly correlate with species identity or habitat preference, highlighting the complexity of ecological interactions and adaptations. The comparison of leaf morphology did reveal significant differences between *H. apiculata* and *H. involuta*, suggesting potential adaptations to specific environmental conditions. The partial overlap observed in the PCA plot indicates considerable intraspecific variation within each species, potentially influenced by environmental factors or genetic processes. The intermediate positioning of some individuals raises questions about possible hybridization or shared genetic ancestry, underscoring the dynamic nature of species boundaries and evolutionary processes.

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