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Research article

A Preliminary Study on Morphological Variations from Wet and Dry Microhabitats of *Hyophila involuta* (Pottiaceae, Bryophyta): A Case Study from Chiang Mai Province, Northern Thailand

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Abstract The morphological variations of a cosmopolitan moss, *Hyophila involuta* (Hook.) A. Jaeger, are reported here using t-test to distinguish 2 distinct forms of ecotypes (humid and arid forms). Three replicates each from 145 collections from 27 places in Doi Inthanon National Park, Doi Suthep-Pui National Park, and Chiang Dao Wildlife Sanctuary, Chiang Mai province, northern Thailand were examined. Eleven gametophytic characters were measured such as stem height, branching, stem diameter, leaf size, leaf blade thickness, size of median and basal laminal cells, length of innermost perichaetial leaf, and length of archegonia. Of these, 10 characters, viz. stem height, leaf size, stem diameter, branching, length of basal laminal cells, length of innermost perichaetial leaf, and length of archegonia were significantly different among populations. Other additional features of the two different ecotypes of *H. involuta* were recorded and discussed including leaf apices, innermost perichaetial leaf apices, marginal teeth, hyaline nodules, and number of cortical and medullary central strand cells.

Keywords: Bryophytes, Chiang Mai, Morphometry, Pottiaceous moss, Wet and dry microhabitats

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INTRODUCTION

Hyophila involuta (Hook.) A. Jaeger (cement moss), an acrocarpous moss in the family Pottiaceae, is a common species which is widely distributed throughout tropical and subtropical regions of the world (Zander, 1993). It can be found at various elevations and in different habitats such as deserts, humid soil, wet rocks, and stream banks of waterfalls and is also found on manmade concrete structures in urban habitats (Eddy, 1990; Li et al., 2001; and Deora & Deora, 2017).

The distinctive characters of the species described in several taxonomic treatments and floras include spatulate-lanceolate leaves with serrulate to denticulate leaf margins that are strongly involute when dry, bulging-mamillose upper laminal cells, well-differentiated leaf bases, costae with two well-developed stereid bands in cross section, stems with a well-differentiated central strand, and cylindrical eperistomate capsules (Gangulee, 1972; Eddy, 1990; Zander 1993; Li et al., 2001; Virendra & Reesa, 2009; Costa, 2015). During our excursions in Doi Suthep-Pui National Park, we found an interesting humid ecotype of cement moss with large stems, up to 5 cm height including 4–5 (8) branches, and vegetative leaf length up to 4 mm. In contrast, the plants growing in dry areas had smaller stems (about 1 cm height, usually unbranched and leaf length up to 2.5 mm). After carefully examination with many literatures reported this species from different part of the world and also comparison with several identified herbarium specimens deposited at the CMUB Herbarium collected from various places in Chiang Mai province, northern Thailand, we did not find any one who described arid and humid form of *H. involuta*. Many literatures cites here including the taxonomic monographs, revisions, and floras such as Gangulee (1972), Saito (1975), Noguchi (1988), Eddy (1990), Zander (1993), Li et al. (2001), Allen (2002) Costa (2016) and etc., usually reported only common morphology of the local plants of *H. involuta* but did not compare size of gametophytes and their sexual structures from wet and dry areas. So, this study aims to preliminarily investigate morphological differences of the cosmopolitan moss, *H. involuta* from dry and wet areas using a morphometric method. This study will provide a new insight on plant structures particularly from the different ecotypes.

MATERIALS AND METHODS

Plant collection

Samples of cement moss (145 packets) were collected from two distinct microhabitats including aquatic microhabitats (shaded-wet rocks in streams or waterfalls, Figure 1C) and terrestrial microhabitats (open-dry soils or rocks, Figure 1H) (see Appendix). The specimens were identified using the taxonomic keys in Gangulee (1972), Eddy (1990), Zander (1993), Li et al. (2001), Virendra & Reesa (2009) Costa (2015), and Deora & Deora (2017). All specimens were then collected in paper bags and dried at room temperature. The vouchers were deposited in the CMUB herbarium of the Biology Department, Faculty of Science, Chiang Mai University, Thailand.

Morphometric study

Three replicate gametophytes and sporophytes of *H. involuta* from the 145 collections from 27 populations were selected and rehydrated in the laboratory. Morphological characters of each gametophyte (150 gametophytes from 7 wet areas and 200 gametophytes with sporophytes from 20 dry areas, see Supplement) were examined. Stem height, branching (number of branches and the length of branches) were measured. Stem diameter, leaf size, leaf blade thickness, size of median and basal laminal cells were determined using a light-transmission microscope, and the number of sporophytes was also recorded. We also considered other gametophytic characters such as leaf apices (apiculus, apical leaf shaped, and number of apical and marginal teeth), hyaline nodules (a group of swollen, color less, thin-walled cells, well differentiate in the epidermis of stem near the leaf insertion) and number of cortical and medullary cells in cross section of stems, which were noted as additional characters.

Data analysis

Statistical analysis was performed using SPSS statistic program version 17 (SPSS Inc., Chicago, USA). Significantly different means of each character between dry and wet areas were identified by the T-test at the 99.99% confidence level.

RESULTS

Independent samples T-test of gametophytic characters of humid and arid forms of *H. involuta*

T-test of 10 gametophytic characters of *H. involuta* grown on wet and dry habitats showed significantly different among populations (Table 1). Generally, gametophytes from the dry habitats were mostly small with average stem height and stem diameter of 6.5 mm (Figures 1E, 1D af, 1G, and 3#1–20) and 180.0 μm (Figure 2B), respectively. Most plants were unbranched and also had small leaves (approximately 0.7×2.2 mm). Gametophyte stems usually produced sporophytes (Figure 1E, 1G, and 1H, and Figures 3#1–20). In contrast, most gametophytes from the wet habitats were larger, with average stem height and stem diameter values of about 28.5 mm (Figures 1A, 1B, 1D hf, and Figures 5#21–27) and 323.3 μm (Figure 2A), respectively. Up to 6 branches (average 2.2) per stem were found (Figures 1A, 1B, and Figures 5#21–27). The plants also had bigger leaves up to 4 mm long (Figures 1F hf, 4#27, and 5#21–27), but they rarely produced sporophytes. The length of basal laminal cells of the humid form were longer than those of the arid form (Figure 2I), approximately 13.3×56.1 μm (vs 13.6×38.6 μm in the arid form, Figure 2J). Further, innermost perichaetial leaves of humid forms were different from arid forms by having larger size (approximately 2.1–2.6 mm long), ovate-lanceolate with abruptly narrow acuminate leaf apices and shortly excurrent costae (Figures 6G–K), whereas smaller size of innermost perichaetial leaves (approximately 1.2–1.4 mm long), lingulate with obtuse-rounded leaf apices and the costae ending below leaf apex in several cells were found in arid form (Figures 6A–F). The length of archegonia of humid form were longer than the archegonia of arid form (Figure 2D), approximately 0.8–1.0 mm long (vs 0.45–0.56 mm long in arid form, Figure 2E).

Table 1. Independent samples T-test of 10 characters of *H. involuta* grown on wet and dry habitats from Chiang Mai province, northern Thailand (*, $P < 0.0001$).

Characters	Habitats	N	Mean $\pm$ SD	t
1. Stem height (mm)	wet	150	28.5 $\pm$ 7.3	34.2*
	dry	200	6.5 $\pm$ 3.3	
2. Width of leaves (mm)	wet	150	0.8 $\pm$ 0.2	6.6*
	dry	200	0.7 $\pm$ 0.1	
3. Length of leaves (mm)	wet	150	3.0 $\pm$ 0.4	17.8*
	dry	200	2.2 $\pm$ 0.4	
4. Branching number	wet	150	2.2 $\pm$ 1.3	14.9*
	dry	200	0.4 $\pm$ 0.8	
5. Length of branches (mm)	wet	150	8.9 $\pm$ 5.7	16.9*
	dry	200	0.7 $\pm$ 1.8	
6. Length of innermost perichaetial leaves (mm)	wet	30	2.4 $\pm$ 0.2	19.7*
	dry	50	1.4 $\pm$ 0.2	
7. Length of archegonia (mm)	wet	150	0.89 $\pm$ 0.09	38.3*
	dry	150	0.52 $\pm$ 0.06	
8. Stem diameter (μm)	wet	150	323.3 $\pm$ 44.4	31.7*
	dry	200	180.0 $\pm$ 38.3	
9. Width of basal cells (μm)	wet	150	13.3 $\pm$ 2.1	-1.2
	dry	200	13.6 $\pm$ 2.4	
10. Length of basal laminal cells (μm)	wet	150	56.1 $\pm$ 12.4	14.4*
	dry	200	38.6 $\pm$ 9.7	

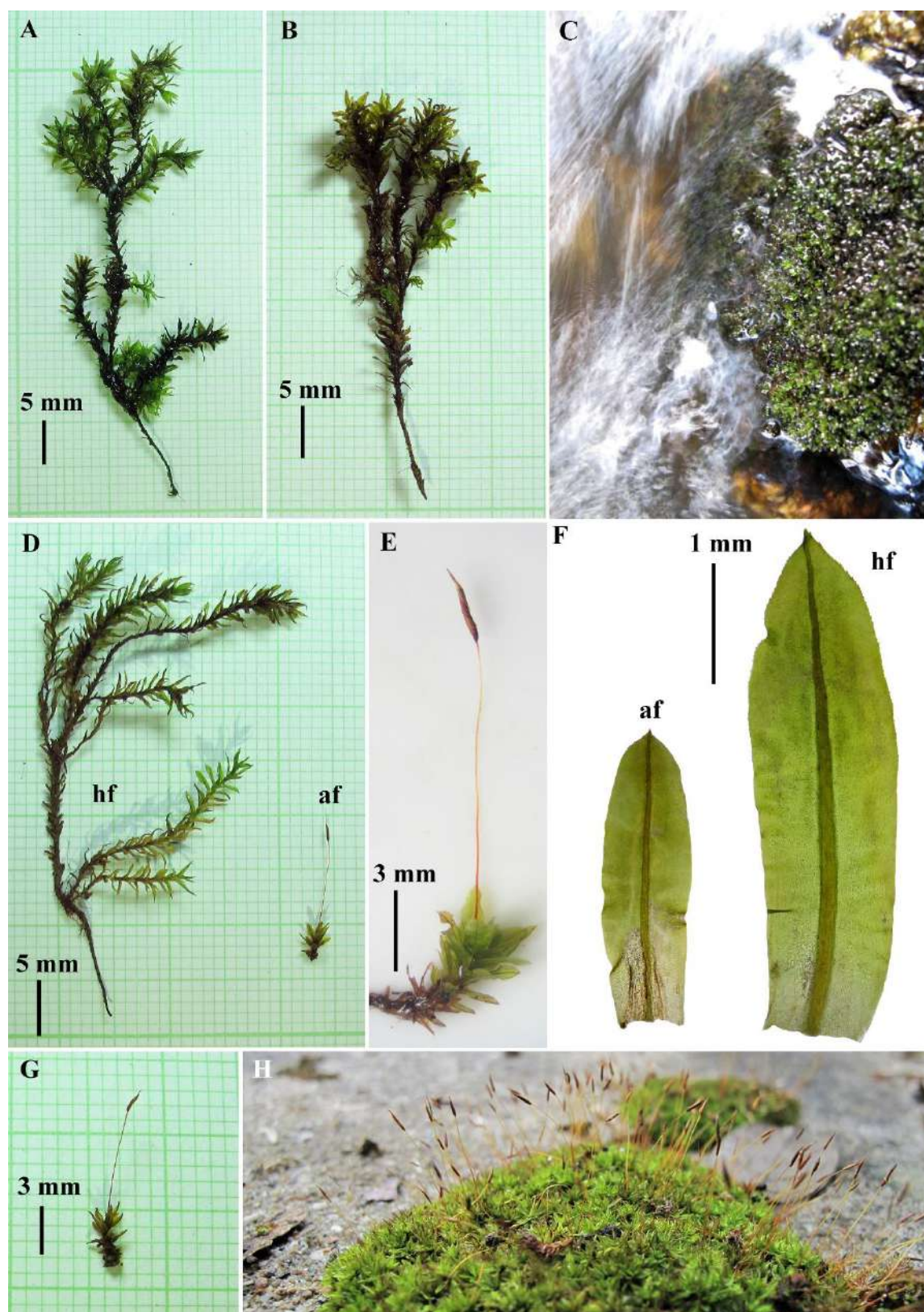


Figure 1. Ecotypes of *Hyophila involuta* (Hook.) A. Jaeger (Pottiaceae), humid form (hf) and arid form (af). A and B: Humid form habits. C: Wet habitat at Huay Kaew Falls, ca. 415 m, Doi Suthep-Pui National Park. D: Comparison of habit size from different habitats. E: and G: Arid form habits. F: Comparison of leaf size from different habitat. H: Dry habitat (opened soil) at Sirindhorn Observatory area, ca. 850 m, Doi Suthep-Pui National Park.

Additional characters on morphological variations of *H. involuta*

Among additional characters, the leaf apices of *H. involuta* from humid areas were narrowly acute and cuspidate with strongly serrate margins, cusps multicellular, 2–3 cells (Figures 2F, H) while those of the plants from dry areas were broadly acute to obtuse and remotely denticulate (Figures 2C, G).

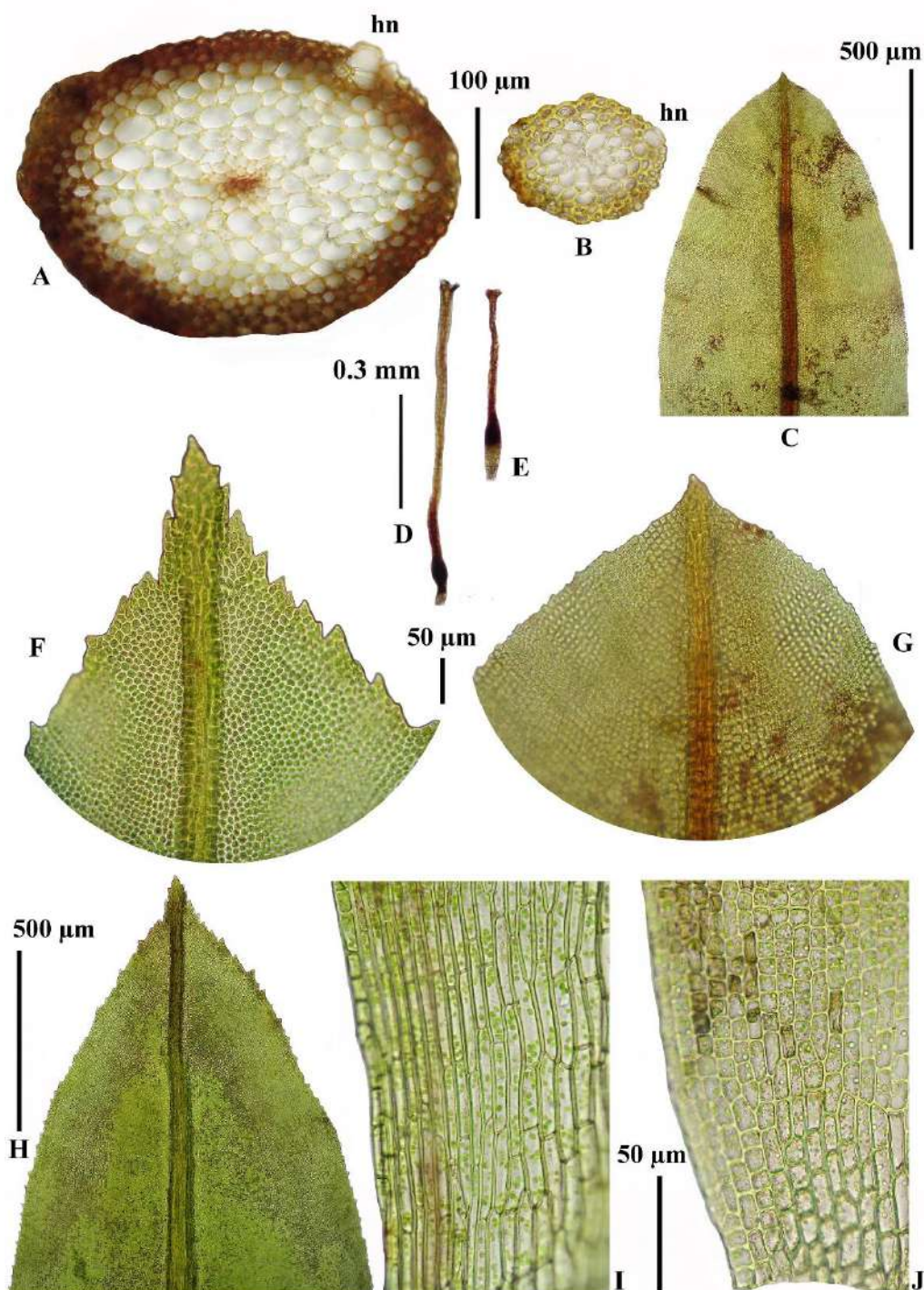


Figure 2. Character variations of *H. involuta* grown on different microhabitats.

A: Stem cross sections showing broader cortical layer and well-differentiated central strand and hyaline nodules (hn) in humid form. B: Stem cross section of arid form. C and H: Leaf apices of arid form (C) and humid form (H). D: Archegonia of humid form (D) and arid form (E). F and G: Apical laminal cells and their denticulae variations of a humid form (F) and an arid form (G). I and J: Comparison of basal laminal cells of humid form (I) and arid form (J).

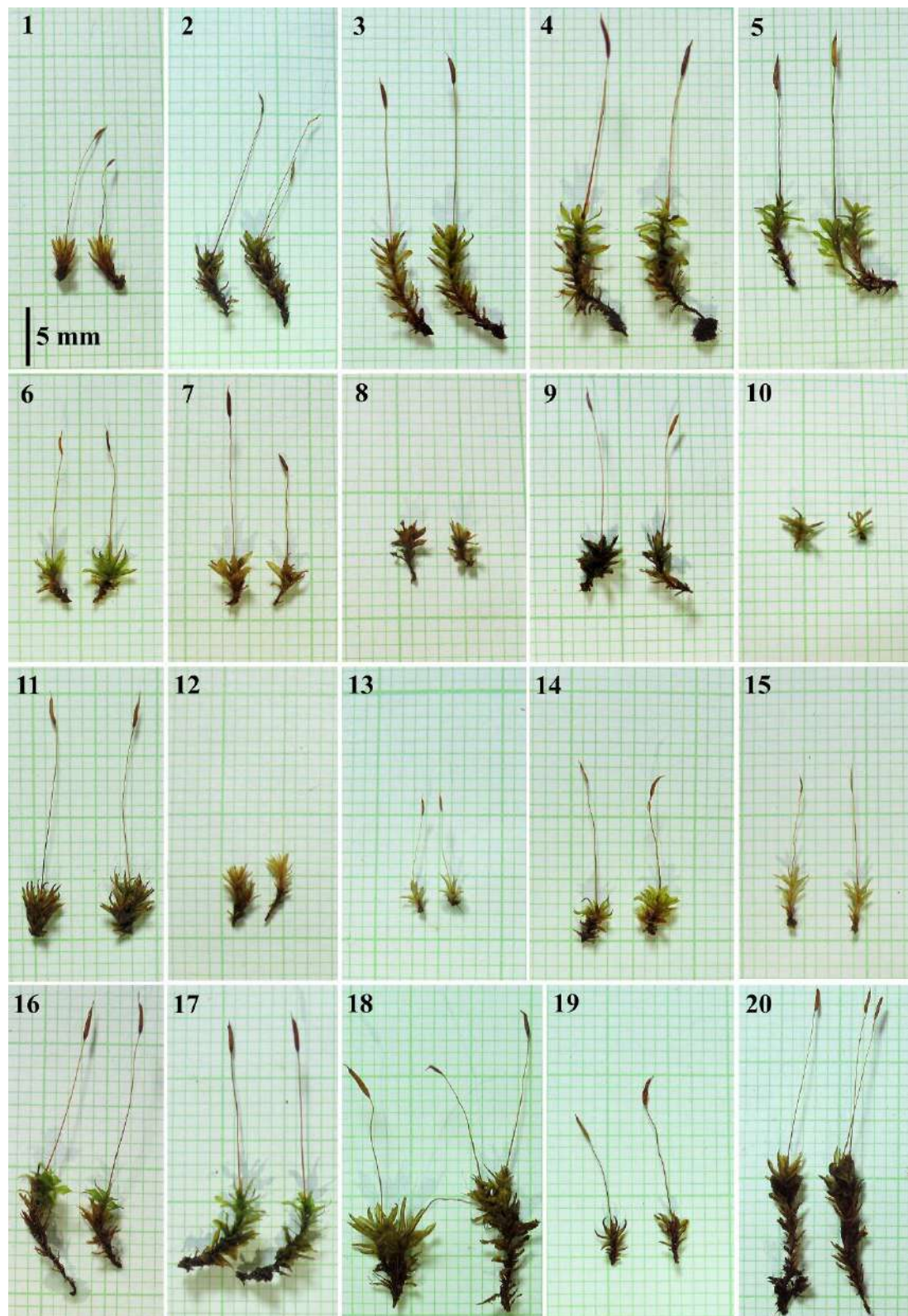


Figure 3. Arid form habits of *H. involuta* collected from several places in Chiang Mai Province; numbering codes of locations are listed in supplement.

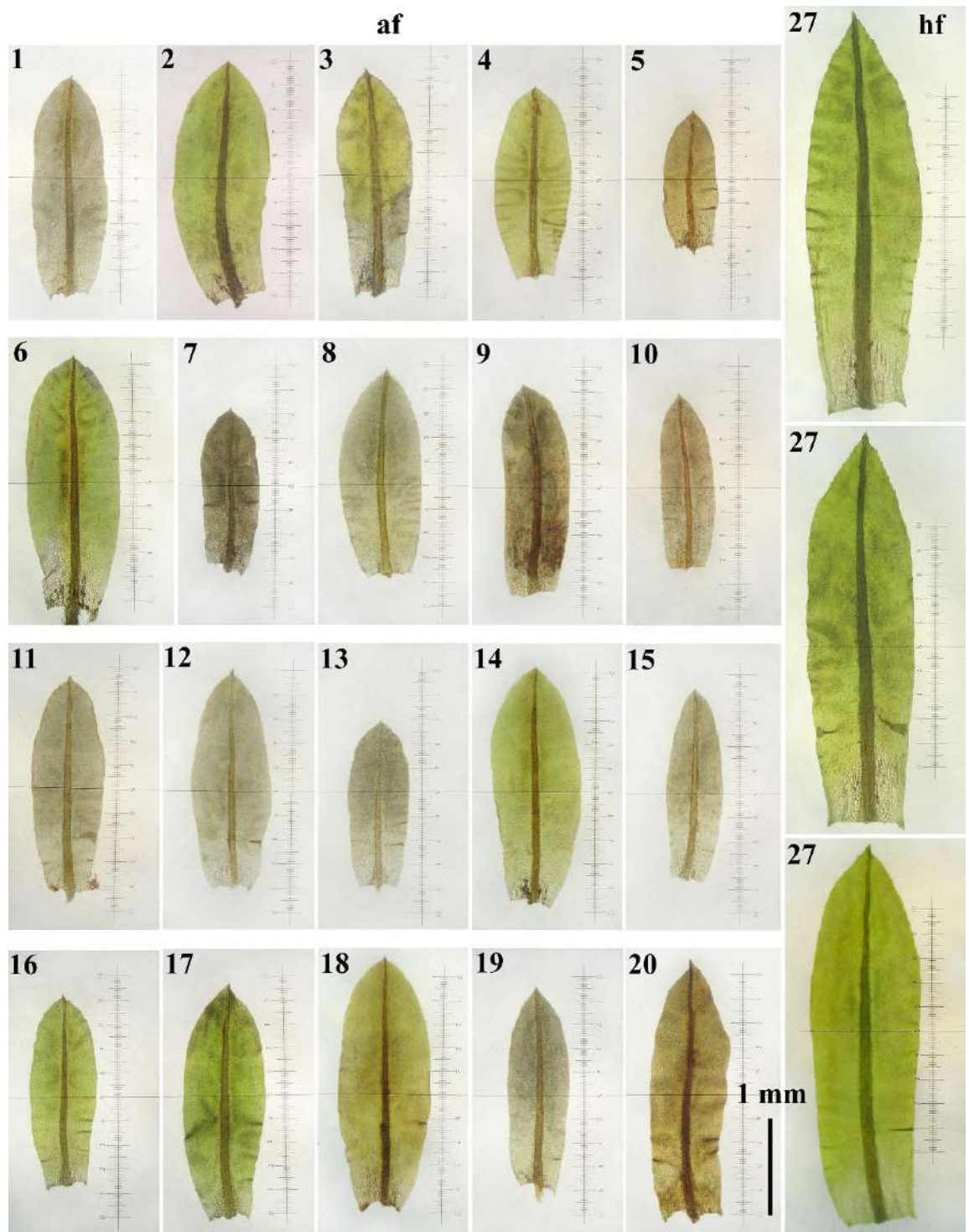


Figure 4. Morphological variations of leaves in *H. involuta* collected from several places in Chiang Mai Province, arid form (af), humid form (hf); numbering codes of locations are listed in supplement.

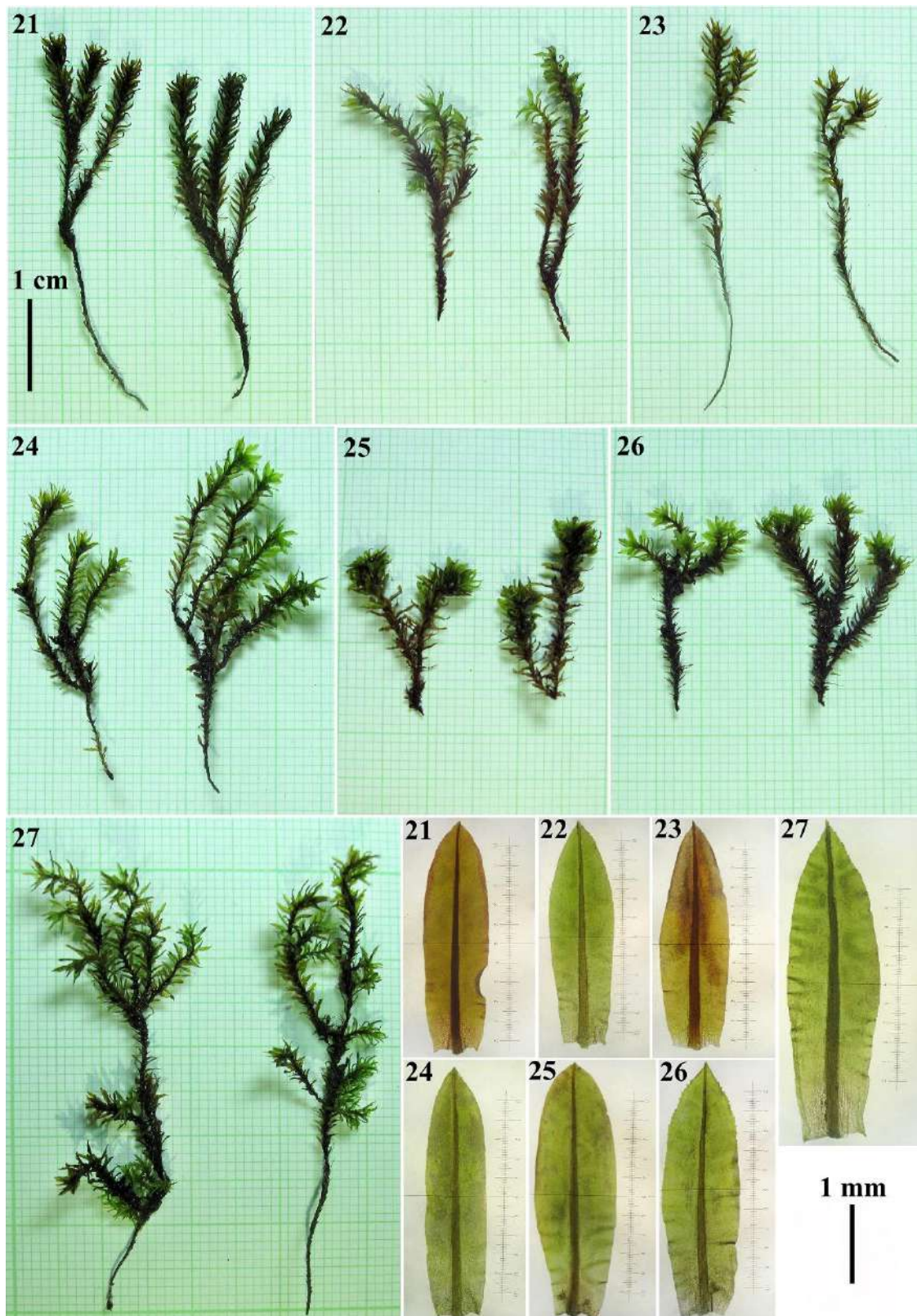


Figure 5. Humid form habits and their leaves of *H. involuta* collected from several places in Chiang Mai Province; the numbering codes of locations are listed in supplement.

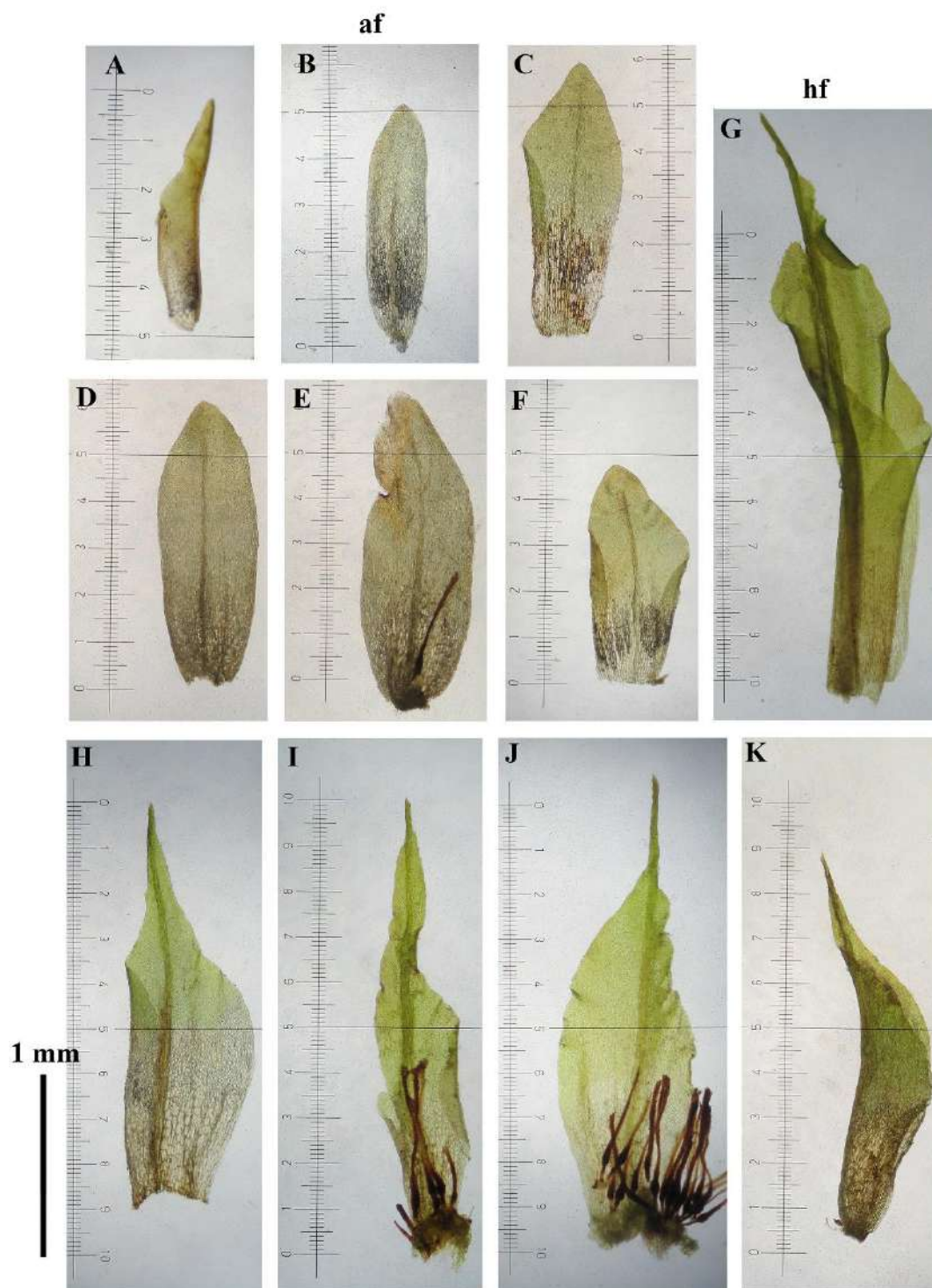


Figure 6. Innermost perichaetial leaves of *H. involuta* grown on different microhabitats. A-F: Innermost perichaetia of arid form (af). G-K: Innermost perichaetia of humid form (hf).

Belonging to the species of genus *Hyophila* that have involute leaves, bulging mamilllose cells, and entire leaf margins. *Hyophila involuta* can be easily separated by its remotely denticulate to serrate upper leaf margins. However, this worldwide species can be confused only with *H. propagulifera* Broth., which has finely serrulate to entire leaf margins and produced numerous gemmae. *Hyophila propagulifera* differs from *H. involuta* by its leaf cross sections with bulging mamilllose on both surfaces, gemmae obovoid, smooth on surfaces, and was known from Japan and China (Brotherus, 1899; Saito, 1975; Noguchi, 1988; Li et al., 2001), whereas bulging mamilllose only on adaxial

surfaces and smooth on abaxial surfaces of laminal cells in cross section (Figures 7A-D), and tuberclose to echinate gemmae were found in *H. involuta* (Figure 7E).

The plants described as *H. angustiuscula* Baumgartner & Dixon, which had been treated as synonym of *H. involuta* (Eddy, 1990: 199). *Hyophila angustiuscula* differs from normal *H. involuta* by only slightly larger laminal cells than usual and the involute leaves were found even in the fresh state; the enlarged laminal cells might be the habitat modification found in submerged (Eddy, 1990: 199). This is probably being the early evidence for the humid ecotype of *H. involuta*, which had been reported from other wet places. Unfortunately, other characters of humid form did not discuss in his hand book of Malesian mosses. By the way, our study showed insignificantly on the size and thickness of upper laminal cells, which were about 5–7 μm diameter in both humid and arid ecotypes (Figures 7A-D); but we found the difference of leaf cross sections, humid form showed slightly wider costae about 50–75 μm whereas narrower costae about 25–35 μm were found in arid form (Figures 7C-D). Other distinctive different characters of humid form found in our study were significantly larger inner perichaetial leaves with narrowly acuminate apices and excurrent costae of inner perichaetia, whereas smaller inner perichaetial leaves with obtuse rounded and costae vanishing below apex in inner perichaetia of arid form; archegonia of humid forms were longer neck cells than arid form; basal laminal cells of humid form were different longer than arid form; stems in cross section of humid form showed more cell layers both in cortex and central strands (4–7 cortically and 6–8 medullary central strand, Figure 2A) than those arid-form stems that had 2–3 cortical layers and 3–5 layers of central strand (Figure 2B); the water conducting hyaline nodules were well differentiated on the epidermis and outer cortex of humid-form stems with 2–3 groups of large hyaline cells (usually 1–3 cell layers, Figure 2A, hn), comparing in the arid-form stems, which had only 1 smaller hyaline cell (Figure 2B, hn).

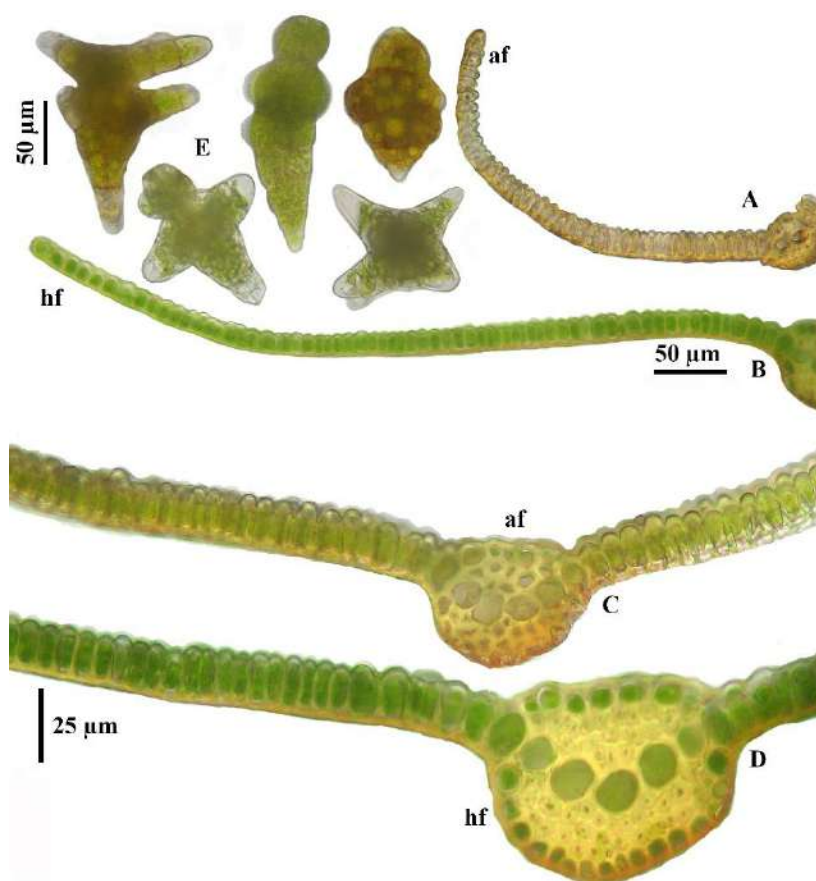


Figure 7. *Hyophila involuta*, leaves in cross section. A and C: Leaves in cross section of arid form (af). B and D. Leaves in cross section of humid form (hf). E. Gemmae of humid form.

DISCUSSIONS

Results from this study showed distinct characters of *H. involuta* growing on dry and wet areas. Stem length, stem diameter and length of basal laminal cells are major characters distinguishing between the plants from dry and wet areas while other characters such as branching, leaf size, innermost perichaetial leaves, and length of archegonia can be used as supporting evidences. Our results are different from a previous study on a pottiaceous moss, *Syntrichia laevipila* complex in which sporophyte characters (sporophyte size) were suggested as a main factor indicating variation among populations (Gallego et al., 2005).

The innermost perichaetial leaves are smaller than normal leaves, highly unstable lingulate to lanceolate, and having obtuse-rounded to acute apices found in many species of *Hyophila*, e.g. *H. apiculata* M. Fleisch., *H. javanica* (Nees) Brid., and *H. involuta* (Li et al., 2001; Eddy, 1990: 199–201). These are also variable in the costae of the innermost perichaetia, which are vanishing far below apices in several row of cells in *H. javanica* but lacking a costa in *H. apiculata* (Eddy, 1990: 199–201). It is interesting to note that *H. involuta* in wet habitats having ovate-lanceolate innermost perichaetia (up to 2.5 mm long) with abruptly narrow acuminate leaf apices, and having shortly excurrent costae, were recorded at first time for this study.

Different characters found in this species reflect its ability to grown in various habitats. *Hyophila involuta* can produce both spores and asexual propagules. This might be being a good strategy for this cosmopolitan species, resulting in its worldwide distribution everywhere, especially in lowland habitats. In this study, we found an abundance of stalks producing claviform-stellate gemmae clustered on the leaf axils of the humid form, but stalks were reduced in the arid form, while sporophytes were abundantly produced in the arid form. Even though morphological adaptations in bryophytes have been little documented, reduced or minute gametophytic forms without branching could be an important strategy to cope with dryness (Proctor et al., 2007; Stark et al., 2007). In a study of Deora & Deora (2017), only 5 species of mosses including *H. involuta* could be found in the Thar Desert, the hottest and driest region on the Indian subcontinent. The plants of *H. involuta* in this area were very small (only 2 mm height). In our study, the smallest size (2–4 mm height) arid forms can be found on rocks and soils in open-dry habitat at Sirindhorn Observatory area, Doi Suthep-Pui National Park in deciduous, dipterocarp-oak, seasonal, hardwood forest, ca. 850 m elevation. Forming crowded colonies with short and dense stems could help this moss survive in arid areas (Mägdefrau, 1982; Grace, 1995). Moreover, leaves that are always involute when dry, is considered to be another adaptation to prevent water loss from the plants. In this study, we also found hyaline cells which are probably involved in the poikilohydric property of *H. involuta*, to enhance water absorption and maintain moisture inside stems when exposed to dry air. The combination of desiccation tolerance and poikilohydry in several mosses is a highly evolved strategy of morphological adaptation to minute size, which enables growth on limited moisture substrates (Proctor & Tuba, 2002).

In contrast, in humid forms, *H. involuta* increased stiffness of the stems by forming a hypodermal sterome that can support long stems that stand upright or resist rapid water flow. This adaptation has been observed in giant terrestrial mosses for example *Dendroligotrichum dendroides* (Brid. ex Hedw.) Broth., Polytrichaceae (Frenzke et al., 2011). Aquatic mosses like *Fontinalis dalecarlica* Bruch & Schimp., Fontinalaceae also have stiff stems to resist rapid currents, while *F. flaccida* Renaud & Cardot has flaccid stems that cannot withstand strong water flow, resulting in this species occurring only in lakes and pools (Glime, 2017). Furthermore, many studies found two kinds of stems in mosses according to their water conducting pathways (Buch, 1947; Schofield, 1981; Huttunen et al., 2018). Ectohydric stems rely mainly on water conduction along the cortex or external surface of the plants whereas endohydric mosses conduct water inside the central strand. Our results revealed that stems of humid forms matched well with endohydric stems by having well differentiated 6–8 medullary central strand cells unlike forms in the dry places that have less differentiate central strand cells with shorter stems. Moreover, the stems of humid forms of *H. involuta* also had clustered hyaline nodules. It could help water conduction and act as branch primordia (buds) to produce

more branches. This structure can help other mosses adapt to their habitats such as *Fissidens* spp., Fissidentaceae, (Iwatsuki & Pursell, 1980; Pursell, 2007). It is interesting to note that Thai specimens found on wet rocks in the waterfalls have more differentiate axillary hyaline nodules than those of *H. involuta* from other parts of the world, which were reported absent or weakly differentiate hyalodermis (Zander, 1993; Eddy, 1990, Li et al., 2001; Saito, 1975; etc.). In our field works, we found *H. involuta* on wet rocks along waterfalls at elevation between 350–1,240 m, growing intermingled with *Fissidens* spp. that have well differentiated hyaline nodules such as *F. crispulus* Brid. var. *crispulus*, *F. geminiflorus* Dozy & Molk. *F. subangustus* M. Fleisch., and *F. javanicus* Dozy & Molk., while in dry places *H. involuta* can be found on soils, rocks, and concretes in lowland, deciduous forest, near ridged mountains, and manmade habitats at elevation between 350 m to 2,500 m of disturbed places near summit of Doi Inthanon (Mt.) along with *F. zollingeri* Mont., *F. biformis* Mitt., *F. ceylonensis* Dozy & Molk., and *F. wichurae* Broth. & M. Fleisch., which are smaller size, have well differentiate hyaline nodules and shared habitats with *H. involuta*.

Evidence for cryptic speciation has been reported in other mosses (Shaw, 2001). In this case, we found that the two forms from different microhabitats are morphologically differentiated enough to separate *H. involuta* into two forms of ecotypes (humid and arid forms). However, molecular information is still needed to confirm separation of the two forms as distinct species.

CONCLUSION

The morphological variations of *H. involuta* grown on wet habitats were larger gametophytic characters than dry habitats such as stem height, leaf size, stem diameter, innermost perichaetia, and archegonia. Number of branching and length of branches in wet habitats were up to 6 branches and 15 mm long (vs unbranched in dry habitats). Innermost perichaetial leaves in wet habitats were ovate-lanceolate with abruptly narrow acuminate leaf apices and shortly excurrent costae (vs lingulate with obtuse-rounded leaf apices and the costae ending below leaf apex in several cells found in dry habitats). T-test of independent samples separated *H. involuta* populations into 2 groups according to wet and dry habitats and distinguished 2 distinct forms of ecotypes (humid and arid forms).

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Supplement

Two hundred arid form gametophytes and sporophytes of *H. involuta* (100 pockets belonging to 20 populations of dry areas) grown on soils, rocks, in forests and urban concretes in Chiang Mai Province, Northern Thailand were listed below:

Code No.	Places	Elevation (m)	Specimens examined (CMUB Herbarium)	No. of gametophytes examined (plants)
1	Chiang Mai University	350	28 August 2009: <i>Printarakul</i> 2171; 6 January 2010: <i>Printarakul</i> 2580; 5 January 2012: <i>Printarakul</i> 5214 (3 pockets)	10
2	Wachirathan Falls, Doi Inthanon National Park	660	11 April 2020: <i>Printarakul</i> 11042020_34, 11042020_35, 11042020_36 (3 pockets)	10
3	Siriphum Falls, Doi Inthanon National Park	1,240	11 April 2020: <i>Printarakul</i> 11042020_10, 11042020_11, 11042020_12, 11042020_16, 11042020_17 (5 pockets)	10
4	Kew Mae Pan, Doi Inthanon National Park	2,160	23 July 2007; <i>Nathi</i> 194; 22 November 2019: <i>Jampeatong</i> 32; 11 April 2020: <i>Printarakul</i> 11042020_20, 11042020_21, 11042020_22, 11042020_23 (6 pockets)	10
5	Summit of Doi Inthanon, Doi Inthanon National Park	2,565	11 April 2020: <i>Printarakul</i> 11042020_23A, 11042020_24, 11042020_25 (3 pockets)	10
6	Huay Kaew Falls, Doi Suthep-Pui National Park	410	12 May 2012: <i>Printarakul</i> 5293, 5342; 20 August 2012: <i>Printarakul</i> 5477; 5 February 2020: <i>Printarakul</i> 05022020_2, 05022020_3, 05022020_4 (6 pockets)	10
7	Mae Sa Falls, Doi Suthep-Pui National Park	420	23 November 2012: <i>Printarakul</i> 5717, 5723; 29 November 2012: <i>Printarakul</i> 5755 (3 pockets)	10
8	Tad Mork Falls, Doi Suthep-Pui National Park	500	5 December 2012: <i>Printarakul</i> 5821, 5841, 5856 (3 pockets)	10
9	Mork Fa Falls, Doi Suthep-Pui National Park	560	12 December 2012: <i>Printarakul</i> 5905, 5936, 5984 (3 pockets)	10
10	Huay Pha Lad, Doi Suthep-Pui National Park	620-750	6 January 2011: <i>Printarakul</i> 3415; 14 June 2011: <i>Printarakul</i> 3917, 3937; 22 June 2011: <i>Printarakul</i> 4026, 4068 (5 pockets)	10
11	Doi Suthep, Doi Suthep-Pui National Park	480-1,050	1 December 2005: <i>Printarakul</i> 14; 3 June 2009: <i>Printarakul</i> 1220; 6 September 2011: <i>Printarakul</i> 4660, 4712; 14 September 2011: <i>Printarakul</i> 4731, 4738; 21 September 2011: <i>Printarakul</i> 4783; 13 March 2020: <i>Printarakul</i> 13032020_5, 13032020_6, 13032020_7 (10 pockets)	10
12	Monthathan Falls, Doi Suthep-Pui National Park	730	27 November 1997: <i>Rattanayan & Santanachote</i> 207; 15 June 2006: <i>Polboonsri</i> 32; 27 July 2011: <i>Printarakul</i> 4262, 4269, 4301 (5 pockets)	10
13	Sirindhorn Observatory areas, Doi Suthep-Pui National Park	850	17 September 2005: Manachit 163, 164; 12 December 2005: Manachit 263; 20 December 2010: <i>Printarakul</i> 3382; 13 March 2020: <i>Printarakul</i> 13032020_1, 13032020_2, 13032020_3, 13032020_4 (8 pockets)	10
14	Ru See (hermit) Cave, Doi Suthep-Pui National Park	1,150	13 March 2020: <i>Printarakul</i> 13032020_8, 13032020_9, 13032020_10 (3 pockets)	10
15	Puping Palace, Doi Suthep-Pui National Park	1,300	8 August 2012: <i>Printarakul</i> 5431; 1 August 2017: <i>Printarakul</i> 7166; 13 March 2020: <i>Printarakul</i> 13032020_11, 13032020_12, 13032020_13 (5 pockets)	10

Code No.	Places	Elevation (m)	Specimens examined (CMUB Herbarium)	No. of gametophytes examined (plants)
16	Bahn Mohng (village) Doi Pui, Doi Suthep-Pui National Park	1,400	13 March 2020: <i>Printarakul</i> 13032020_14, 13032020_15, 13032020_16 (3 pockets)	10
17	Summit of Doi Pui, Doi Suthep-Pui National Park	1,685	13 March 2020: <i>Printarakul</i> 13032020_18, 13032020_19, 13032020_23, 13032020_24, 13032020_25, 13032020_26, 13032020_27, 13032020_28, 13032020_29, 13032020_30 (10 pockets)	10
18	Doi Mon Long, Doi Suthep-Pui National Park	1,430	6 November 2012: <i>Printarakul</i> 5554; 13 November 2012 : <i>Printarakul</i> 5632 ; 29 December 2019 : <i>Printarakul</i> & <i>Adulkittichai</i> 2 (3 pockets)	10
19	Nature trail to Doi Luang Chiang Dao, Chiang Dao Wildlife Sanctuary	1,100-2,100	11 February 1995: <i>Allen</i> Y77, Y78; 11 August 1995: <i>Allen</i> Y100; 20 October 1995: <i>Gardner</i> Y40; 21 October 1995: <i>Gardner</i> Y59; 27 October 2017: <i>Printarakul</i> 7113, 7116; 9 February 2020: <i>Printarakul</i> 09022020_7; 10 February 2020: <i>Printarakul</i> 10022020_6, 10022020_7 (10 pockets)	10
20	Doi Sam Pee Nong, Chiang Dao Wildlife Sanctuary	1,800	11 March 1995: <i>Allen</i> Y91, Y92, Y96 (3 pockets)	10

One hundred and fifty humid form gametophytes of *H. involuta* (45 pockets belonging to 7 populations of wet areas) grown on wet rocks in streams and water falls in Chiang Mai Province, Northern Thailand were listed below:

Code No.	Places	Elevation (m)	Specimens examined (CMUB Herbarium)	No. of gametophytes examined (plants)
21	Wachirathan Falls, Doi Inthanon National Park,	687	11 April 2020: <i>Printarakul</i> 11042020_26, 11042020_27, 11042020_28, 11042020_29, 11042020_30, 11042020_31, 11042020_33 (7 pockets)	20
22	Sirithan Falls, Doi Inthanon National Park	812	11 April 2020: <i>Printarakul</i> 11042020_1, 11042020_2, 11042020_3, 11042020_4 (4 pockets)	20
23	Pha Dok Seaw Falls, Doi Inthanon National Park	1,128	11 April 2020: <i>Printarakul</i> 11042020_6, 11042020_7, 11042020_8 (3 pockets)	20
24	Siriphum Falls, Doi Inthanon National Park	1,279	11 April 2020: <i>Printarakul</i> 11042020_13, 11042020_14, 11042020_15, 11042020_18 (4 pockets)	20
25	Huay Kaew Falls, Doi Suthep-Pui National Park	417	5 February 2020: <i>Printarakul</i> 05022020_9, 05022020_10, 05022020_11, 05022020_12 (4 pockets)	21
26	Monthathan Falls, Doi Suthep-Pui National Park	730	16 May 2006: <i>Phrompa</i> 6; 24 January 2011: <i>Printarakul</i> 3456; 5 February 2020: <i>Printarakul</i> 05022020_5, 05022020_6, 05022020_7, 05022020_8 (6 pockets)	28
27	Mahidol Falls, Doi Suthep-Pui National Park	1,100	20 August 2009: <i>Printarakul</i> 1947, 1949; 25 August 2009: <i>Printarakul</i> 2004, 2005, 2012, 2030; 31 March 2020: <i>Printarakul</i> 31032020_1, 31032020_2, 31032020_3, 31032020_4, 31032020_5, 31032020_6, 31032020_7, 31032020_8, 31032020_9, 31032020_10, 31032020_11 (17 pockets)	21