

Morphology and Nodal Anatomy of Philippine Endemic *Hornstedtia conoidea* Ridl. (Zingiberaceae)

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ABSTRACT

The morphology and nodal anatomy of *Hornstedtia conoidea* Ridl., a wild Philippine endemic ginger, are herein described based on ocular and microtechnique observations. *Hornstedtia conoidea* is an erect plant reaching 3 meters tall; rhizome 28–35 cm long, 2–2.5 cm thick; leaves distichous, lanceolate, 55–63 cm and 11–13 cm; inflorescence spindle-shaped, 10–13 cm long 3–4 cm wide, covered with involucre bracts; infructescence enclosed by the enlarged bracts, dehiscent, with fruits measuring 12.5–14 cm long by 4–5 cm wide. Nodal anatomy of rhizome (young, middle-aged, old) showed the presence of collateral vascular bundles, endarch xylem differentiation, atactostele, unilacunar-multitraces nodes consisting of one leaf gap, multi leaf traces and having a single-layer of epidermis; also present is an endodermoidal layer consisting of cambium-like cells, the latter is a diagnostic character of Zingiberaceae.

INTRODUCTION

Gingers are monocotyledonous species belonging to family Zingiberaceae which are considered the largest among the eight families of order Zingiberales. They are particularly distributed in South and Southeast Asia (Wu and Larsen, 2000; Kress *et al.*, 2002; and Nontasit *et al.*, 2015). The family is recognized by its aromatic distichous leaves with open sheaths and fleshy rhizome (Larsen and Larsen, 2006). Gingers have various essential oils which are traditionally used as herbal medicines and are commonly cultivated for their use as spices, drinks, dyes and vegetables (Larsen and Larsen, 2006; Nontasit *et al.*, 2015).

Among the important and edible species of ginger is *H. conoidea* commonly known as “panaon, pinoon and panon (Bisaya)”, “tagbak (Mandaya)” and “puso-puso (Tagalog)” (Barbosa *et al.*, 2016; Barbosa *et al.*, 2018; Acma and Mendez, 2018a,b). The genus *Hornstedtia* is characterized by a rigid spindle-shaped inflorescence composed of many sterile bracts (Larsen *et al.*, 1999; Poulsen, 2006; Lamb *et al.*, 2013). The species was first collected in the Philippines at Cuernos Mountains, Dumaguete, Negros Oriental by Ridley on 1909 and is categorized as Philippine endemic.

The ripe seeds of *H. conoidea* are edible and eaten

by local people of Bukidnon, Surigao and Davao, Philippines which were claimed to be good remedy for stomach ache and diarrhea. The rhizomes are for fever, chills and as condiments and leaves as condiments, and have potentials in nutraceutical and drug industries (Bua-in and Paisooksantivatana, 2009; Barbosa *et al.*, 2016; Acma and Mendez, 2018a). However, despite of being a Philippine endemic and having high potential value, the morpho-anatomical aspects of *H. conoidea* are poorly known. Moreover, no information is available on its nodal anatomy – a significant taxonomic character used in the classification of higher vascular plants particularly in various family of angiosperms (Docot *et al.*, 2016).

MATERIALS AND METHODS

Hornstedtia conoidea samples collected from Musuan, Maramag, Bukidnon, Philippines were used for this study. They were placed in plastic bags and directly processed at the Natural Science Research Center (NSRC) of Central Mindanao University. Plant organs like inflorescence, infructescence, leaves, stem, rhizome and seeds were preserved in 70% ethyl alcohol.

1. Morphological Examination

Based on fresh specimens, the gross morphological characteristics of the roots, rhizome, leaf, inflorescence, flowers, infructescence, fruits and seeds were

qualitatively and quantitatively documented.

2. Nodal Anatomical Examination

Free hand sectioning technique by Amoroso and Amoroso (1994) was employed. The nodal anatomical descriptions were obtained from the rhizome which were categorized into: a. young- from the first emergence of the leaf, b. at middle-aged- the area between the young and old parts, and c. old- at the area with hardened parts (Cutler, 2008). The rhizome was cut using razor blade at specific points.

Fixation technique by Battersby (2004) was employed with solution of 90 mL 70% ethyl alcohol, 5 mL glacial acetic acid and 5% formalin. The rhizomes were soaked in the fixative solution for a minimum of 24 hours. Thereafter, the rhizomes were dehydrated slowly in order not to destroy the delicate tissues. This was done gradually in a series of alcohol solutions of increasing strengths (diluted using distilled water, accordingly). The rhizomes were first soaked in 50% ethyl alcohol for 2 hours; then in 70% for at least 12 hours, and in 85, 90 and 100%, each for 1 hour.

The staining and mounting technique followed that of Ruzin (1999) in which sections were stained for 2 to 24 hours in safranin solution. Excess stain was

washed out for a few seconds in running water. Good sections (those of proper thickness) were fixed in 70% ethyl alcohol for at least 30 minutes and then, were washed briefly in water. The sections were dehydrated consecutively in 80 and 90% ethyl alcohol (30 shakes each) for about 10 minutes in each solution. The sections were washed with water and then, excess water was removed. The sections were stained in safranin for 30 minutes and were washed with 95% alcohol. Then, these were cleared through the alcohol-xylene series: 25, 50, 75 and 100% xylene (30 shakes each). The sections were counterstained with fastgreen for few seconds and were mounted on the glass slide using eukit mounting medium.

RESULTS

1. Morphological Description of *H. conoidea*

The roots are adventitious, fleshy, 15–20 cm long by 0.2–1 cm thick, brown, stilt and rough. The rhizome is 28–35 cm long by 2–2.5 cm wide, coarse, elongate and growing horizontally just above the soil surface or arising above the soil on stilt roots and with the spreading habit form clumped. The pseudostem (or false stem) formed by overlapping leaf sheaths arise from rhizome, gives height of the plant that reaches to three meters (Fig. 1). Each leaf sheath is terminated at



Figure 1. (A) Habitat of *Hornstedtia conoidea*; (B) clumped main rhizome (MR), branching rhizome (BR), leaf sheath (ls), inflorescence (infl), infructescence (infr); (C) detached branching rhizome (BR), and roots (r).

apex and grows upward as narrow stem-like structure bearing 15–32 individual of large leaves.

The leaves are distichous, ascending, 55–63 cm long by 11–13 cm wide; venation simple, parallel; sessile to sub-sessile; base cuneate to obtuse; apex attenuate;

margin entire, yellowish brown, lanceolate, adaxially silky dark green, abaxially light green; midrib yellowish brown, adaxially canaliculate and abaxially protruding; ligule entire, oblong, coriaceous, brownish, with white fine hairs, 1.3–1.8 cm long by 0.5–1 cm wide (Fig. 2).

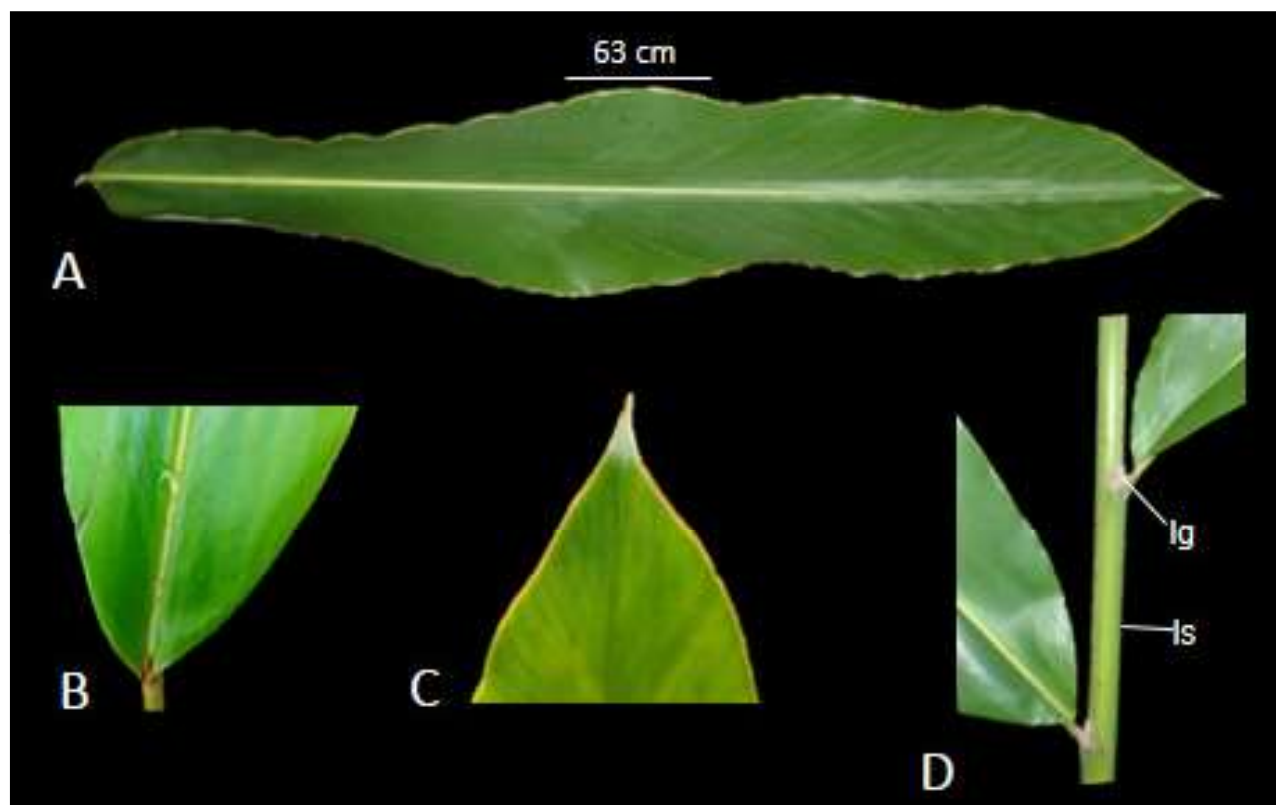


Figure 2. Leaf of *Hornstedtia conoidea*; (A) whole leaf, (B) leaf base, (C) leaf apex and (D) alternate leaf attached to leaf sheaths (ls) and ligule (lg).

Inflorescence is short, erect, coriaceous, spindle-shaped, arising from the rhizome along the soil near the base of the leafy shoot, bearing 1–2 flowers, 10–13 cm long by 3–4 cm wide, covered with sterile involucre bracts. Peduncle short, 1–1.5 cm long, covered with narrow deltoid scales, submerged in the rhizome. Involucre bracts deltoid to triangular, concave like a boat, 6–7.5 cm long by 1.5–4 cm wide, coriaceous, red, base pale red, striate, tightly overlapping, outer surface with white hairs at the base, inner surface silky, margin entire, apex acute. Bracteoles tubular, pubescent, striate, 5–6 cm long by 1–2 cm wide, open at base, pink to pale red, whitish at base part, translucent, apex acute. Calyx elongated to oblanceolate, translucent and light pink 4.5 cm long by 6 cm wide. Style narrow tubular, white to pinkish, soft, 6 cm long. Stigma very small, protruded, shiny. Corolla lobes oblong, red, 1.5 × 0.8 cm. Labellum oblong, without staminodes, red, margin entire, slightly concave shape resembles boat, apex rounded, 1.6 × 0.8 cm. Anther sac cluster tubular, white, 0.8–1 cm long (Fig. 3).

Infructescence enclosed by the enlarged bracts, cone-shaped, dehiscent, short peduncle covered with brittle brown scales, embedded in the rhizome near the base of the leafy shoot, bearing fertile seeds, 12.5–14 cm long by 4–5 cm wide. Bracts red when young, brown and brittle when mature, 8–10 cm long by 4–5 cm wide. Seeds enclosed with capsule, coated with aril, monocotyledonous, white when young, dark brown to black when matured, 0.2–0.3 cm long by 0.1–0.2 cm wide (Fig. 4).

2. Nodal Anatomical Description of *H. conoidea*

Young Rhizome

Young rhizome possesses collateral arrangement of vascular bundles, sclerenchyma sheath surrounding the vascular bundles and ground tissue is composed of parenchyma tissues. Xylem differentiation is endarch, atactostele type of stele, unilacunar with long, distinct multi-leaf trace, with distinct endodermal layer separating the inner and outer cylinder. There is one layer of epidermis with thin and long trichomes (Fig. 5).

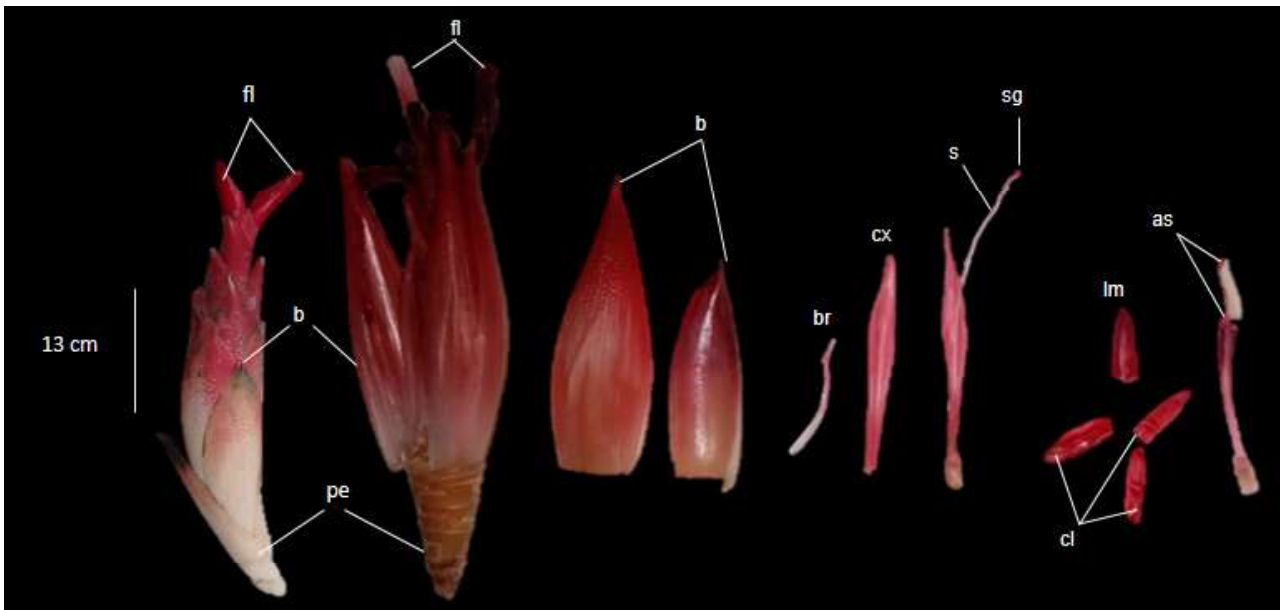


Figure 3. Inflorescence and floral dissection of *Hornstedtia conoidea*; flower (fl), peduncle (pe), bracts (b), bractioles (br), calyx (cx), style (s), stigma (sg), labellum (lm), corolla lobes (cl), anther sac (as).

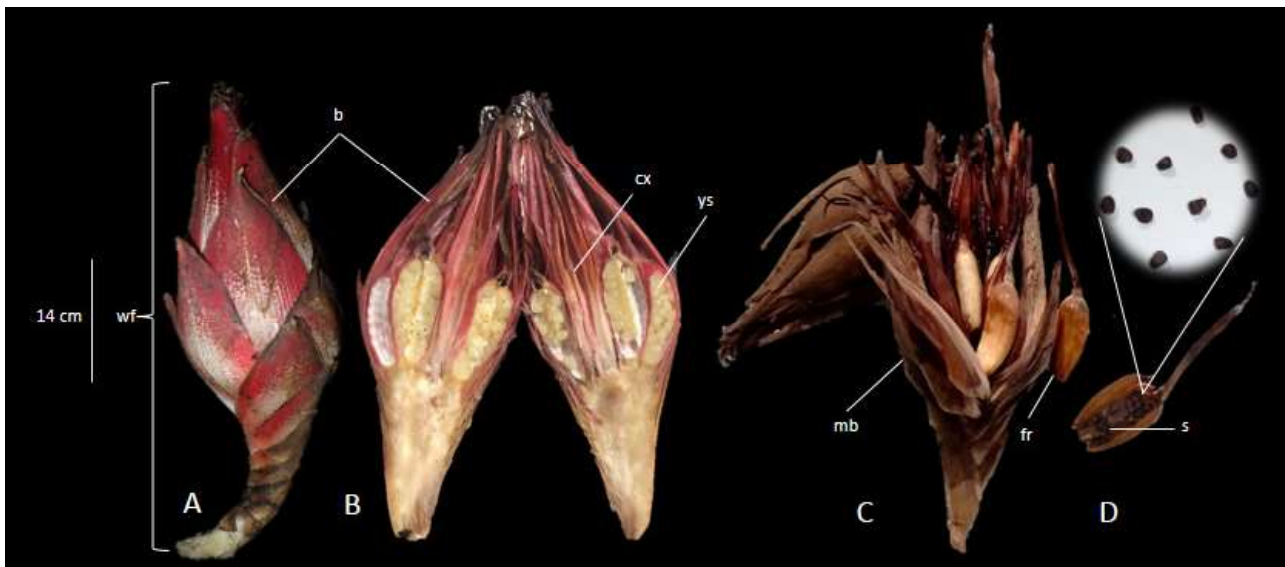


Figure 4. Infructescence of *Hornstedtia conoidea*; (A) with bracts (b); (B) fruit longitudinal section with calyx (cx), young seeds (ys); (C) dissected mature dehiscent fruit, mature bracts (mb), fruit (fr); and (D) dehiscent fruit with seeds (s) inside.

Middle-aged Rhizome

Middle-aged rhizome possesses collateral arrangement of vascular bundles, sclerenchyma sheath surrounding the vascular bundles and ground tissue is composed of parenchyma tissues. Xylem differentiation is endarch, atactostele type of stele, unilacunar with short distinct multi-leaf trace, with distinct endodermoidal layer separating the inner and outer cylinder. There is one layer of epidermis with fine, thick and long trichomes (Fig. 6)

Old Rhizome

Old rhizome possesses collateral arrangement of vascular bundles, sclerenchyma sheath surrounding the vascular bundles and ground tissue composed of parenchyma tissues. Xylem differentiation is endarch, atactostele type of stele, unilacunar with short distinct multi-leaf trace, with very distinct endodermoidal layer separating the inner and outer cylinder. There is one layer of epidermis with fine, thin and short trichomes (Fig. 7).

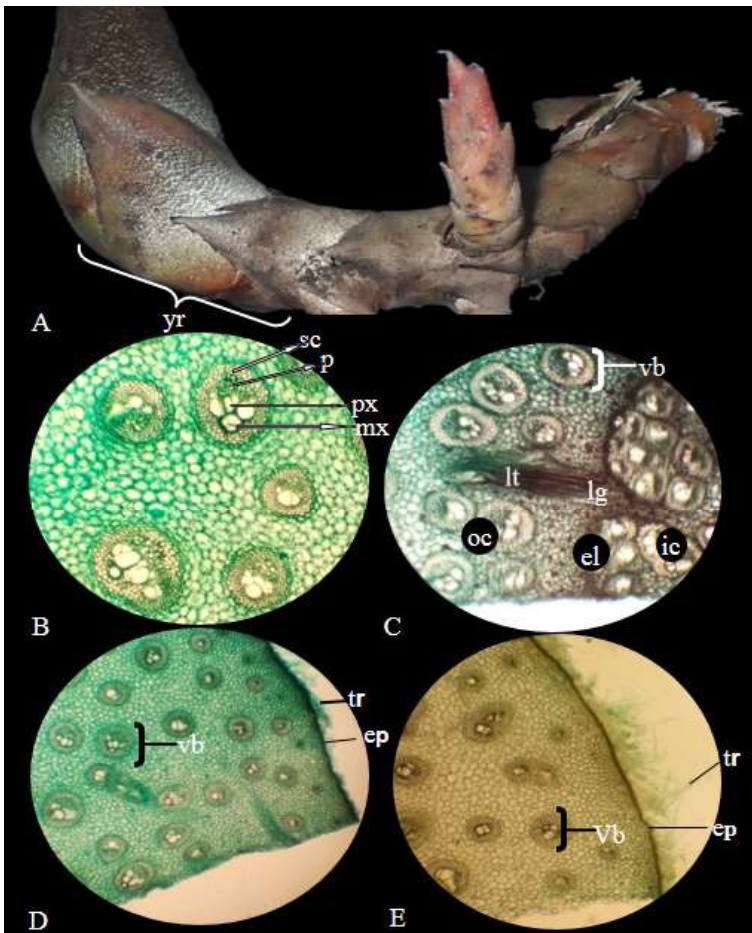


Figure 5. (A) Young rhizome (yr) of *Hornstedtia conoidea*, (B–E) its cross section with vascular bundle (vb), sclerenchyma sheath (sc), phloem (p), protoxylem (px), metaxylem (mx), leaf gap (lg), leaf trace (lt), outer cylinder (oc), endodermoidal layer (el), inner cylinder (ic), trichomes (tr), epidermis (ep) (400X).

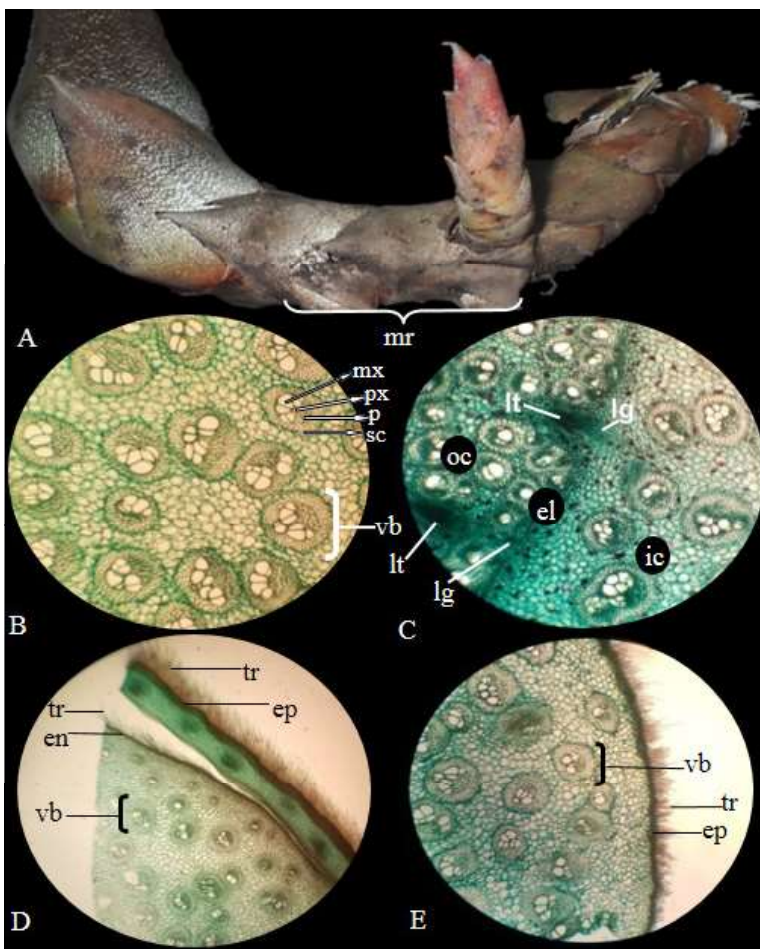


Figure 6. (A) Middle-aged rhizome (mr) of *Hornstedtia conoidea*, (B–E) cross section showing vascular bundle (vb), sclerenchyma sheath (sc), phloem (p), protoxylem (px), metaxylem (mx), leaf gap (lg), leaf trace (lt), outer cylinder (oc), endodermoidal layer (el), inner cylinder (ic), trichomes (tr), epidermis (ep) and endodermis (en) (40X).

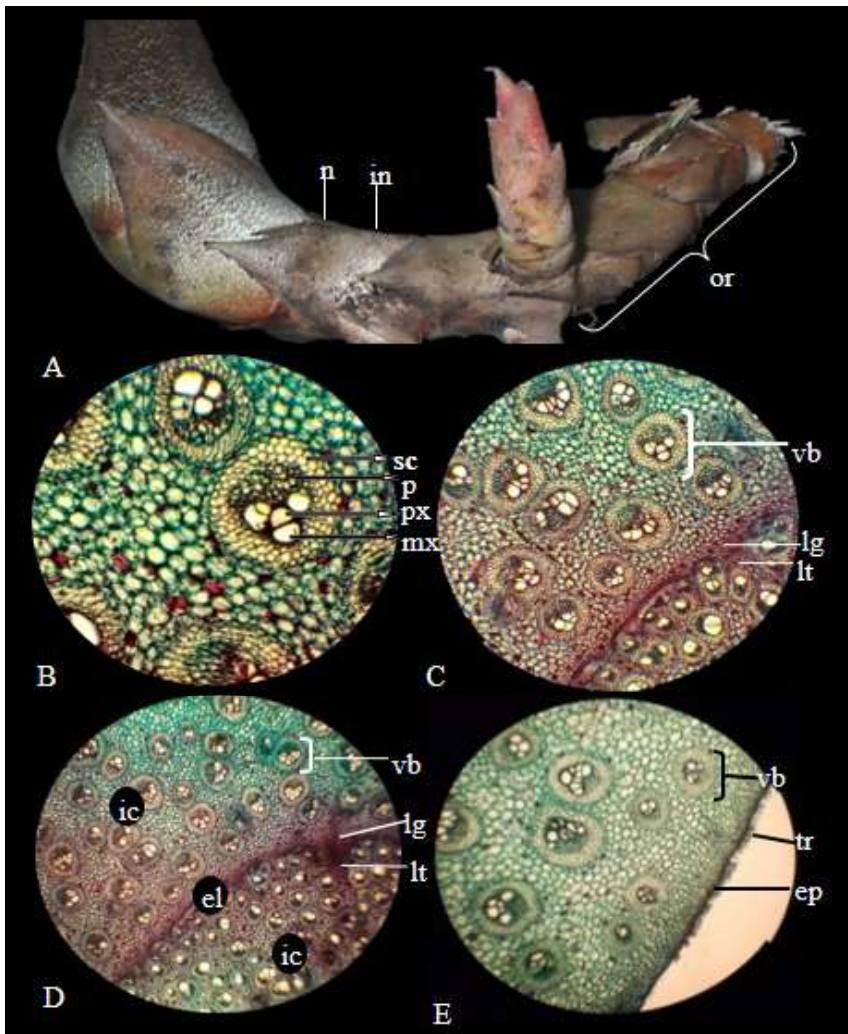


Figure 7. (A) Old rhizome (or) of *Hornstedtia conoidea* its nodes (n) and internodes (in), (B–E) cross section showing vascular bundle (vb), sclerenchyma sheath (sc), phloem (p), protoxylem (px), metaxylem (mx), leaf gap (lg), leaf trace (lt), outer cylinder (oc), endodermal layer (el), trichomes (tr), epidermis (ep) (40X).

DISCUSSION

Most of the ginger species including those of genus *Hornstedtia* possess stilt roots to support their rhizomes. Jansen *et al.* (2016) described the stilt roots of *H. pininga* to support its climbing aerial rhizomes. Similar to *H. conoidea*, it was also emphasized that short stilt roots of *H. scottiana* supports woody branching rhizomes (Ippolito and Armstrong, 1993, Tropical Plants Database, 2021). The rhizomes are possess deltoid-shaped. The involucre bracts are thick, soft and light brown with whitish streaks at the bottom when young, brittle and dark brown when old and closely overlapping with each other. *H. conoidea* is similar to the species *Etingera rosea*, *H. microcheila* by having clumped rhizomes (Naïve, 2017).

The pseudostem (or false stem) formed by overlapping leaf sheaths arises from rhizome, gives height of the plant that reaches as high as three meters. Each leaf sheath is terminated at apex and grows upward as narrow stem-like structure bearing 15–32 individual large leaves. The leaves of *Hornstedtia* are concentrated

in upper of tubular and open leaf sheath (Larsen *et al.*, 1999). *H. microcheila* recorded in the Mt. Mandalagan Range, Patag, Silay City, Negros, Occidental Philippines is one of the species that is morphologically described and also found in the country. *H. microcheila* has reddish midrib and leaf margin (Naïve, 2017) while base on the result of this study *H. conoidea* appears to have yellowish midrib and leaf margin.

The inflorescence arising on separate side shoots from the rhizome, is rigid spindle-shaped and dark red with whitish in the bottom part. Its infructescence is the fertilized inflorescence (composed of several fruits) and dark red with whitish streaks at the bottom part. It bears the seeds which are covered by overlapping bracts (Larsen and Larsen, 2006). The spindle-shaped inflorescence, enclosed densely imbricate involucre bracts often characteristically reticulate or ribbed, absence of staminode and flowers which are red located at the top of inflorescence qualifies this species to belong to the genus *Hornstedtia* (Larsen and Larsen, 2006; Pelser *et al.*, 2011; Acma and Mendez, 2018b). *H. conoidea* is similar to the other gingers like *Hedychium*,

H. scottiana, *Etilingera*, *Amomum*, *Alpinia* by having a distinct dehiscent infructescence (Acma, 2014; Jansen *et al.*, 2016; Tobias *et al.*, 2019).

The xylem and the phloem make up the vascular tissue of a plant termed as vascular bundle. Xylem is commonly known for its function in transporting water and keeping plants hydrated. Phloem is commonly known for its function in carrying important sugars, organic compounds, and minerals around the plant (Purcell, 2020). The collateral vascular bundle in young, middle-aged and old rhizome of *H. conoidea*, is currently one of the basis of identification of a ginger just like of *Etilingera dalican* and *E. philippinense* (Alincastré, 2015). Collateral vascular bundle is characterized as a strand of phloem which is external to the strand of xylem on the same radius. Each vascular bundle of *H. conoidea* is sheathed by layers of sclerenchyma cells and showed endarch xylem differentiation. As such, protoxylem occurs towards the center of the axis and metaxylem occurs towards the periphery of the axis. Such type of differentiation is also called centrifugal. It is arranged in a complex manner called atactostele stele type where in scattered arrangement. Consistently, previous studies of the monocot rhizome is characterized to have endarch xylem differentiation in an atactostele stele type. Results of this study are consistent with the reports of Mauseth (2001) which are characteristics of monocotyledons.

Young, middle-aged and old rhizomes of *H. conoidea* showed inner and outer cylinders separated by endodermoidal layer. On the other hand, monocots

are also characterized by the absence of secondary differentiation. However, unlike in other monocots, the rhizome of ginger possesses a characteristic only certain monocots have, that is the presence of a special meristematic layer along with the endodermoidal layer and this layer consists of cambium-like cells (Nair, 2013). The presence of endodermoidal layer is important feature in rhizome development, the groups of xylem and phloem are formed along this layer while those of inner and outer layer vascular tissues become filled with starch grains (Nair, 2013; Docot *et al.*, 2016). These unique features are also found in ginger species namely *Leptosolenia haenkei* C. Presl and *Vanoverberghia sepulchrei* Merr. (Docot *et al.*, 2016).

As shown in Table 1, young and middle-aged rhizomes of *H. conoidea* possess unilacunar multitraces and old rhizome possesses unilacunar single trace nodal anatomy which signify they are primitive. Nodal region is composed of a vascular strand that extends between the vascular cylinder and the base of leaf called leaf trace and a parenchymatous region between the main vascular cylinder and leaf trace called leaf gap. Unilacunar with leaf traces is very common in monocotyledonous plants, however, previous studies showed that multilacunar nodes is also characteristic of monocots (Gupta, 2020; Pandey *et al.*, 1993). Some ginger species, like *Geocharis fusiformis* exhibited the same form of nodal anatomy (Alincastré, 2015). Difference in sizes of leaf traces and texture and sizes of trichomes were distinct in three different ages of rhizomes shown in Table 1. This implies that the differentiation of procambial cells into vascular tissues takes place at different stages of

Table 1. Nodal Anatomy of *Hornstedtia conoidea* Ridl. Young, middle-aged and old rhizomes.

Characters	Rhizome Ages		
	Young	Middle	Old
Arrangement of Vascular Bundles	collateral	collateral	collateral
Ground Tissues Present	sc, p	sc, p	sc, p
Xylem Differentiation	endarch	endarch	endarch
Type of Stele	at	at	at
Form of Nodal Anatomy	um	um	u
Epidermis (no. of layers)	1	1	1
Trichomes	fine, thin, long	fine, thick, long	fine, thick, short
Leaf trace	long	short	short
Endodermoidal Layer	distinct	distinct	distinct

Legend: sc- sclerenchyma sheath, p- parenchyma, at- atactostele, um- unilacunar multi-traces, u- unilacunar

rhizome growth (Nair, 2013).

The cross section of young, middle and old rhizome exhibited one layer of epidermis, collateral vascular bundles, which are enclosed by sclerenchyma cells and with trichomes on its rhizomes. Moreover, anatomical studies of Liu *et al.* (2020) also showed young and old rhizomes on *Zingiber officinale* had one layer of epidermis (Liu *et al.*, 2020) (Table 1).

CONCLUSION

The study has successfully provided new information on morphology and nodal anatomy of *Hornstedtia conoidea* Ridl. It revealed that *H. conoidea* is an erect plant reaching up to 3 meters; rhizome is 28–35 cm by 2–2.5 cm, elongate and growing horizontally; leaves are distichous, 55–63 cm by 11–13 cm and lanceolate; inflorescence spindle-shaped, bearing 1–2 flowers, 10–13 cm by 3–4 cm, covered with involucre bracts; infructescence enclosed by the enlarged bracts, dehiscent, bearing fertile seeds, 12.5–14 cm by 4–5 cm. Nodal anatomy of young, middle-aged and old rhizomes possess collateral vascular bundles, endarch xylem differentiation, atactostele arrangement of vascular bundle, unilacunar-multitraces nodes, and possess special meristematic layer, the endodermoidal layer which is a unique character of Zingiberaceae. And the distinct difference on leaf traces and trichomes in three ages of rhizomes indicates that the differentiation of vascular tissues takes place at different stages of rhizome growth.

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