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Morphological Study of Pseudopeltate Scales in *Davallodes hymenophylloides* and *Wibelia divaricata* (Davalliaceae)

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Abstract We studied the detailed cellular-level structure of the pseudopeltate scales of *Davallodes hymenophylloides* and *Wibelia divaricata* (Davalliaceae). Results revealed that the structures of the pseudopeltate scales are complicated not only in the cellular contents but also in the shield structures. The developmental pattern of the pseudopeltate scale of *D. hymenophylloides* was similar to that of the peltate scales of other Davalliaceae and related ferns, suggesting a close phylogenetic relationship of the two scales.
Key words : Davalliaceae, fern, scale development, scale structure.

Introduction

In leptosporangiate ferns, the rhizomes are usually invested with trichomes, scales, or both as dermal appendages. Scales are present in various taxa and are suggested to have arisen recurrently in the leptosporangiate ferns (Pryer *et al*., 1995). They are various in color and form (*e.g*., thin or thick, clathrate or not, entire or toothed at the margin, acuminate or obtuse at the tip, truncate, round or cordate at the base, basifixed, pseudopeltate or peltate) and are commonly used as taxonomic characters (Kramer and Green, 1990).

Basifixed scales are attached by broad bases, while peltate and pseudopeltate scales, composed of a shield and a stalk, are attached by cylindrical stalks perpendicular to the shields. In the peltate scale, the stalk is inserted near or below the center of the shield, while in the pseudopeltate scale, though it is apparently similar to the peltate scale, the stalk is attached strictly at the base of the shield and the shield proximal to the stalk is cordate and imbricate. Our previous study inferred that peltate and pseudopeltate scales, which are common in several groups of ferns including epiphytes in the broad sense, have an evolutionary linkage to the epiphytic life form in the lineage leading to Davalliaceae and Polypodiaceae (Tsutsumi and Kato, 2008). Our studies also suggested that morphological changes between peltate and pseudopeltate scales may have occurred multiple times, because both types of scales are seen in the same genera in Davalliaceae and Polypodiaceae (Tsutsumi and Kato, 2006, 2008).

Most previous systematic studies have focused on the morphology of the scales and made gross-morphological observations (Nayar *et al*., 1968; Sen *et al*., 1972; Kato, 1975, 1985). Our anatomical study of peltate and pseudopeltate scales in Davalliaceae and related ferns (Tsutsumi and Kato, 2008) revealed that there are variations in the cellular-level and histological features and the degree of stalk insertion into a rhizome concavity. The cells of the central part of the shield around the stalk vary from those containing cytoplasm to those lacking cytoplasm and having thick walls, and to those having dense contents particularly in the exterior zone of the shield. The stalk of some species is attached to the flat rhizome, and other species have stalk deeply inserted into rhizome depressions. The detailed structure of pseudopeltate scales remains yet to be described because the scales are so complicated.

In this study, we report the detailed cellular-

level structure and development of pseudopeltate scales of *Davallodes hymenophylloides* (Blume) M. Kato et Tsutsumi, (previousely called *Araiostegia hymenophylloides* Blume) and the structure of *Wibelia divaricata* (Blume) M. Kato et Tsutsumi (previousely called *Davallia divaricata* Blume) for better understanding the diversity of the scales in Davalliaceae.

Materials and Methods

Sources and vouchers of *Davallodes hymenophylloides* and *Wibelia divaricata* (Davalliaceae) are shown in Table 1. Scales with rhizomes were collected and fixed in FAA (formaldehyde : acetic acid : 50% ethanol = 5 : 5 : 90). To observe scale structure, portions of rhizomes about 5 cm apart from the apex were cut into 10–12-μm-thick sections using a sliding microtome (LEICA SM2000R, Leica, Germany) on a freezing stand (MCR 802R, Komatsu, Kanagawa, Japan). Sections were embedded in a glycerol solution and sealed with nail polish.

To observe the development of scales of *D. hymenophylloides*, apices of rhizomes and leaves were dehydrated in an ethyl alcohol series for approximately 2 h and embedded by HistoResin Plus for 4 d (Leica, Heidelberg, Germany). The embedded materials were cut into 2–5-μm-thick sections with a glass knife on an ultramicrotome (LEICA RM2155, Leica, Vienna, Austria). Sections were stained with safranin, toluidine blue, and orange G (Jernstedt *et al*., 1992).

Results

Anatomy of pseudopeltate scale

Selected serial longitudinal sections of a pseudopeltate scale of *Davallodes hymenophylloides* by freezing microtomy showed that the posterior part of the shield was attached to the posterior side of the stalk but the middle of the stalk (Fig. 1). In a section of a periphery stalk, the structure of the scale looked like a peltate scale with a stalk inserted in a shield (Fig. 1A). In a submedian section on one side, the anterior shield was double layered and the upper shield was a continuation of the posterior shield, which in turn was similar to a peltate structure (Fig. 1B). This structure was caused by a hooked end of the scale base, along which the shield was attached to the stalk (Fig. 2). As mentioned by Tsutsumi and Kato (2008), serial sections showed that the stalk and the central part of the shield comprised cells with cytoplasm and the other part of the shield was one cell thick and

Table 1. Source and voucher of species examined in this study

Species	Source/voucher
Davallodes hymenophylloides (Blume) M. Kato et Tsutsumi	Gunung Salak, Java, Indonesia; *IN89*
Wibelia divaricata (Blume) M. Kato et Tsutsumi	Cibodas, Java, Indonesia; *IN27*

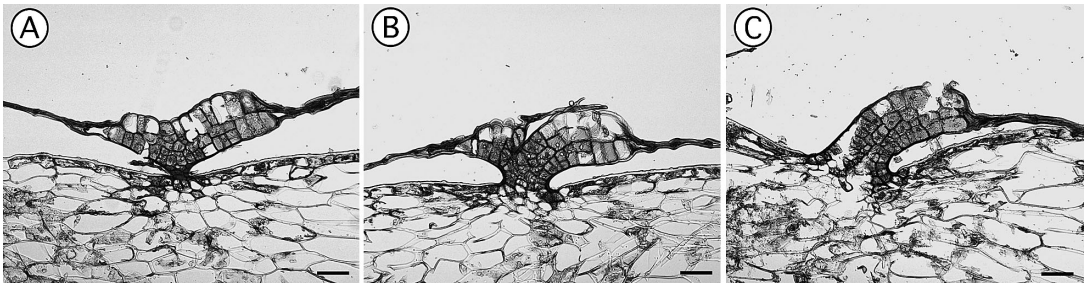

Fig. 1. Selected serial sections of pseudopeltate scale of *Davallodes hymenophylloides*. The rhizome apex is located to right. **A**. Section at periphery of stalk. **B**. Near middle. **C**. Middle. Scale bar = 100 μm.

devoid of cytoplasm. The stalk was short and located in a nearly flat or a very shallow depression of the rhizome.

The pseudopeltate scale of *Wibelia divaricata* was similar to that of *D. hymenophylloides* in that the posterior part of the shield was attached to the posterior side of the stalk except for the middle (Fig. 3). This scale, however, was different from that of *D. hymenophylloides* in that the anterior shield had a posterior projection and was always single layered thorough serial sections in this study (Fig. 3). The stalk was composed of very small cells and prominently sunken in a deep concavity of the rhizome. Cells of the shield near the stalk were dead and consisted mostly of cell walls (Fig. 3; Tsutsumi and Kato, 2008).

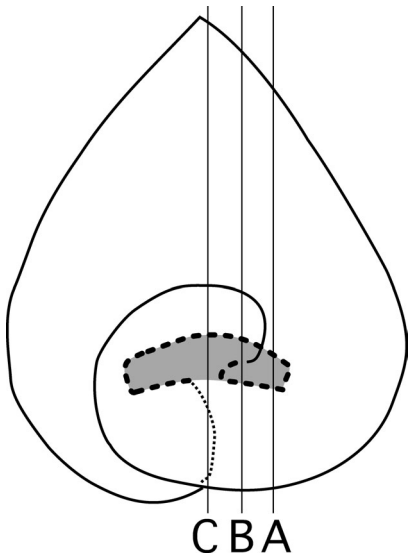

Fig. 2. Diagram of pseudopeltate scale of *Davallodes hymenophylloides*. Lines **A**–**C** indicate sectioning positions in Fig. 1. The stalk is shown in gray, and the connection of stalk and shield is indicated by thick broken line.

Development of Pseudopeltate scale in Davallodes hymenophylloides

The early development of the pseudopeltate scale was quite similar to that of the peltate scales of *Nephrolepis cordifolia* (L.) C. Presl (Lomariopsidaceae) and *Davallia trichomanoides* Blume (Davalliaceae) (Figs. 2C–F and 3 in Tsutsumi and Kato, 2008). One-cell layered scales were produced near the rhizome apex and leaf primordia by oblique and then by transverse cell divisions (Figs. 4A–C). The basal part of the scale, or an initial stalk, underwent cell divisions longitudinally and transversely to become a stalk consisting of multiple rows of cells (Figs. 4D–E). Selected serial sections showed that the cordate posterior part of the shield was formed in the submedian and lateral parts of the stalk by cell divisions of the dorsal cells, which were derived from subdeltoid subbasal cells (Fig. 4E), whereas no or few cell divisions occurred in the middle on the posterior side (Fig. 4D). In the course of scale development, there was no remarkable change in the epidermis and outer cortex of the rhizome (Fig. 4A). It differed from the developmental pattern of *D. trichomanoides*, in which the epidermis and outer cortex of the rhizome around the scale gave rise to deep concavities by differential growth as the stalk elongated (Tsut-

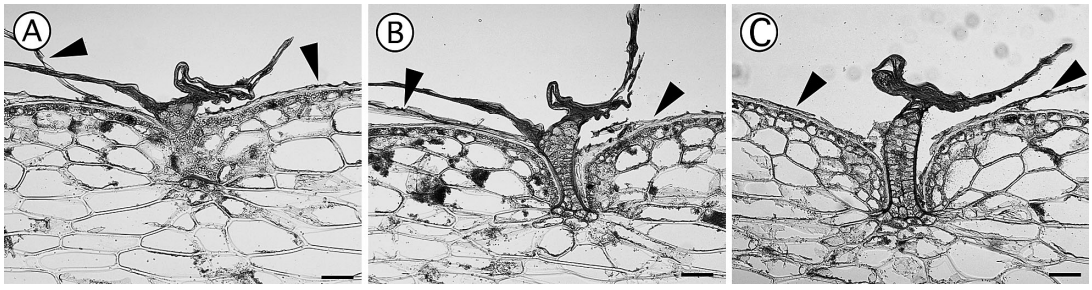

Fig. 3. Selected serial sections of pseudopeltate scales of *Wibelia divaricata*. The rhizome apex is located to right. **A**. Section at periphery of stalk. **B**. Near middle. **C**. Middle. Arrowheads indicate shields of neighboring scales. Scale bar = 100 μm.

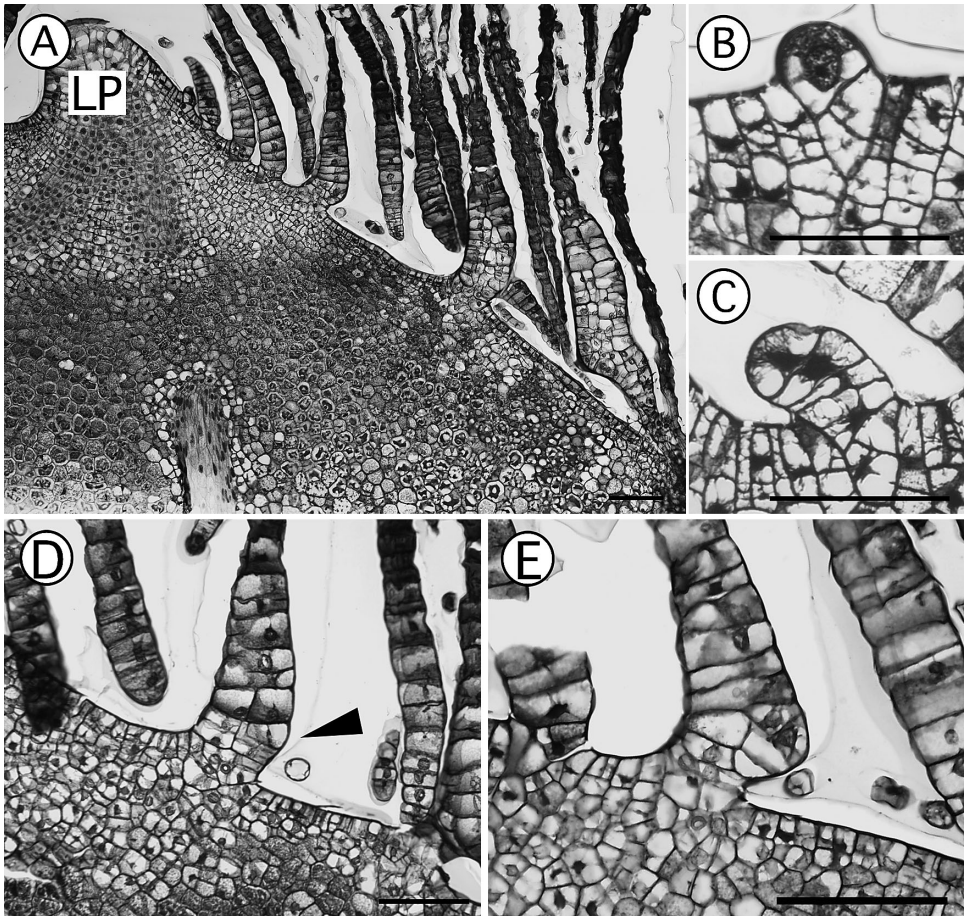

Fig. 4. Longitudinal sections of young pseudopeltate scales at different developmental stages in *Davallodes hymenophylloides*. The rhizome apex and leaf primordium (LP) are located to left. **A**. Different stages of scales. **B**. Enlarged scale initial with oblique division. **C**. Young one-cell layered scale. **D**–**E**. Selected serial sections of more developing scale. **D**. Median section showing absence of subdeltoid subbasal cell (portion marked by solid arrowhead). **E**. Submedian section near edge of stalk, showing subdeltoid subbasal cells. Scale bar = 100 μm.

sumi and Kato, 2008).

Discussion

Our results showed that the cellular-level structures of the pseudopeltate scales are more complicated than those expected by a gross morphological study (Kato, 1975). The pseudopeltate scale of *Davallodes hymenophylloides* had double-layered anterior parts of the shield, the upper of which was caused by the hooked end of the base of the posterior shield. The upper anterior shield covered the stalk and lower anterior shield, and appears to protect the stalk and the shield, whose cells are alive. In *Wibelia divaricata*, the anterior part of the shield was always observed to have a projection at the base. The projection also covered the stalk and may protect the stalk of living cells. In contrast, peltate scales of other Davalliaceae and related ferns had thick-walled shield cells around the stalk, except the scale of *Arthropteris beckleri* (Hook.) Mett. (Tsutsumi and Kato, 2008).

The developmental pattern of the posterior shield of pseudopeltate scales in *D. hymenophylloides* was similar to that of the peltate scales of *Nephrolepis cordifolia* and *Davallia trichomanoides* (Tsutsumi and Kato, 2008). Although there was no developmental data, the upper anterior shield of the pseudopeltate scale may be a result of a prominent growth of one of the posterior shield lobes in association with a hooked base of the shield. It is suggested that the pseudopeltate scales are similar to the peltate scales in developmental mechanisms. It has no inconsistency against the hypothesis that the shifts between peltate and pseudopeltate scales may have occurred multiple times.

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