

## Rhizome Morphology and Patterns of Leaf Production of Secondary Hemiepiphytic *Oleandra pistillaris* (Oleandraceae)

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**Abstract** Secondary hemiepiphytic *Oleandra pistillaris* and *Nephrolepis* are characterized by rhizome dimorphism; the rhizomes are erect and creeping (stolon). The erect rhizome produces leaves in apparent whorls at subequal internodes, but the creeping rhizome of *O. pistillaris* is dorsoventral with variously long internodes, and the creeping stolon of *N. cordifolia* grows without any leaves. It is in contrast to the general monomorphic rhizome with regular phyllotaxy in other life forms of related ferns.

**Key words**: internodes, leaf production, *Nephrolepis*, *Oleandra pistillaris*, rhizome, secondary hemiepiphyte.

### Introduction

Hemiepiphytes grow both epiphytic and terrestrial during their life history, and this life form is among remarkable plant lives, e.g., terrestrial, epiphytic, lithophytic, and aquatic. Hemiepiphytes are classified into two, based on ontogenetic sequences of their life forms; one is primary hemiepiphytes, which grow as epiphytes at the early stage of the life history and later become terrestrial by rooting into the soil (e.g., *Ficus*). The other is secondary hemiepiphytes, which are terrestrial at the early stage of the life history and later become epiphytic by maturity (Putz and Holbrook, 1986). In ferns, some species of *Oleandra* (Oleandraceae), *Nephrolepis* (Lomariopsidaceae), *Colysis*, *Pyrrosia*, *Microsorium* (Polypodiaceae; Tsutsumi and Kato, 2006), and *Trichomanes* (Hymenophyllaceae, treated as hemiepiphytic climber in Dubuisson *et al.*, 2003) are secondary hemiepiphytes.

A molecular phylogenetic tree deduced from *rbcl* and *accD* sequences showed that *Oleandra* (*O. pistillaris* (Sw.) C. Chr. and *O. wallichii* (Hook.) C. Presl) was sister to a clade compris-

ing Davalliaceae and Polypodiaceae (Tsutsumi and Kato, 2006). This group was then sister to a clade of *Arthropteris* clade consisting of climbing *Arthropteris beckleri* (Hook.) Mett., lithophytic *Tectaria phaeocaulis* (Rosenst.) C. Chr. and *Quercifilix zeylanica* (Houtt.) Copel. The clade of these ferns formed a monophyletic clade together with secondary hemiepiphytic *Nephrolepis acuminata* (Houtt.) Kuhn, *N. cordifolia* (L.) C. Presl and climbing *Lomariopsis spectabilis* (Kunze) Mett. Then, this clade was sister to some Dryopteridaceae. These phylogenetic relationships infer that secondary hemiepiphyte of *Oleandra* and *Nephrolepis* was of independent origin. Based on a character evolution of life forms using the phylogenetic tree, a hypothesis was proposed that obligate epiphytic Davalliaceae and Polypodiaceae evolved from climbers or secondary hemiepiphytes. It inferred that secondary hemiepiphyte is an intermediate life form between terrestrial plant and obligate epiphyte in the group examined (Tsutsumi and Kato, 2006).

In general, vascular plants have leaves on the stems in a consistent pattern over space and time, forming the regular architecture of the plant to

achieve high productivity (Steeves and Sussex, 1989). In contrast, secondary hemiepiphytic *Oleandra pistillaris* shows anomalous rhizome morphology; the lengths of internodes are various and they sometimes reach as much as 2 m, resulting in an irregular phyllotaxy. To reveal the unusual architecture of the secondary hemiepiphytic *Oleandra pistillaris* and another secondary hemiepiphytic *Nephrolepis cordifolia*, we examined their shoot apices using scanning electron microscopy.

### Materials and Methods

Samples of *Oleandra pistillaris* were collected from Gunung (=Mt.) Salak, Java, Indonesia (vouchers; *IN28*, *IN39*, *IN96*, *IN102*) and Gunung Arab, Sabah, Malaysia (*ML116*). Rhizomes were cut at 5 mm apart from the apex, and scales were carefully removed. For scanning electron microscopy (SEM), the molds of fresh materials were created by a dental impression material (Provil Novo, Heraeus Kulzer, Hanau, Germany). Based on the molds, positive replicas were created using epoxy resin (Williams *et al.*, 1987). In case of fixed materials by FAA (formalin : acetic acid : 50% ethyl alcohol, 5 : 5 : 90, v/v/v), samples were dehydrated in an ethyl alcohol and isoamyl acetate, and then dried in a critical point dryer (HCP-2, Hitachi, Tokyo, Japan). The replicas or the dried materials were coated with platinum-palladium using a sputter coater (Ion Sputter E-1030, Hitachi, Tokyo). Observations were made using a JSM-820S SEM (Jeol, Tokyo) at 5–15 kV. For the observation of *Nephrolepis cordifolia*, a plant cultivated in the Botanical Garden, Graduate School of Science, the University of Tokyo, was used. Vouchers are deposited in the Herbarium of National Museum of Nature and Science (TNS).

### Results

The rhizome of the secondary hemiepiphytic *Oleandra pistillaris* was dimorphic, comprising a long, creeping rhizome and erect, relatively short

rhizome branches (Fig. 1A–C). The long, creeping rhizome was dorsiventral, branched on the lateral sides, and produced roots on the ventral side and a few leaves on the dorsal side. The lengths of the internodes were various ranging from ca. 1 cm to 150(–240) cm. The erect rhizome elongated to some extent without leaves after branching and produced units of pseudoverticillate leaves at nearly constant intervals. The erect rhizome was rarely branched and had few roots. In a greenhouse, when the rhizome apex was damaged, another rhizome apex in the same individual happened to change into the other rhizome type.

The number of leaf primordia per rhizome apex in the creeping rhizomes examined was various; apparently no primordium ( $N=5$ ), one ( $N=2$ ), and three primordia ( $N=1$ ) (Fig. 1F–I). In the erect rhizomes, no leaf primordium was sometimes observed immediately after branching from the creeping rhizome ( $N=3$ ). In more or less elongated rhizomes, several leaf primordia were produced on the apex without recognizable phyllotaxy ( $N=8$ ) (Fig. 1D–E).

In the secondary hemiepiphytic *Nephrolepis*, two distinct shoots were also reported; long, creeping stolons with roots and buds, and short erect shoots with fascicled leaves, roots and stolons (Holtum, 1955). In *N. cordifolia*, the short shoot generated fascicled leaves and stolons. In contrast, the stolon had no leaf, and regularly generated resting buds alternately on the lateral sides, which may grow into a short shoot or a stolon. By gross morphological observations, however, it could not be determined which shoot type is produced from a resting bud. The growth of the dormant buds irregularly occurred, and thus, the length of the bud-derived stolon between short shoots varied from 2.5 cm to 173 cm.

### Discussion

In the secondary hemiepiphytic *Oleandra pistillaris*, the erect rhizomes always generated pseudo-whorled leaves in the later stage after

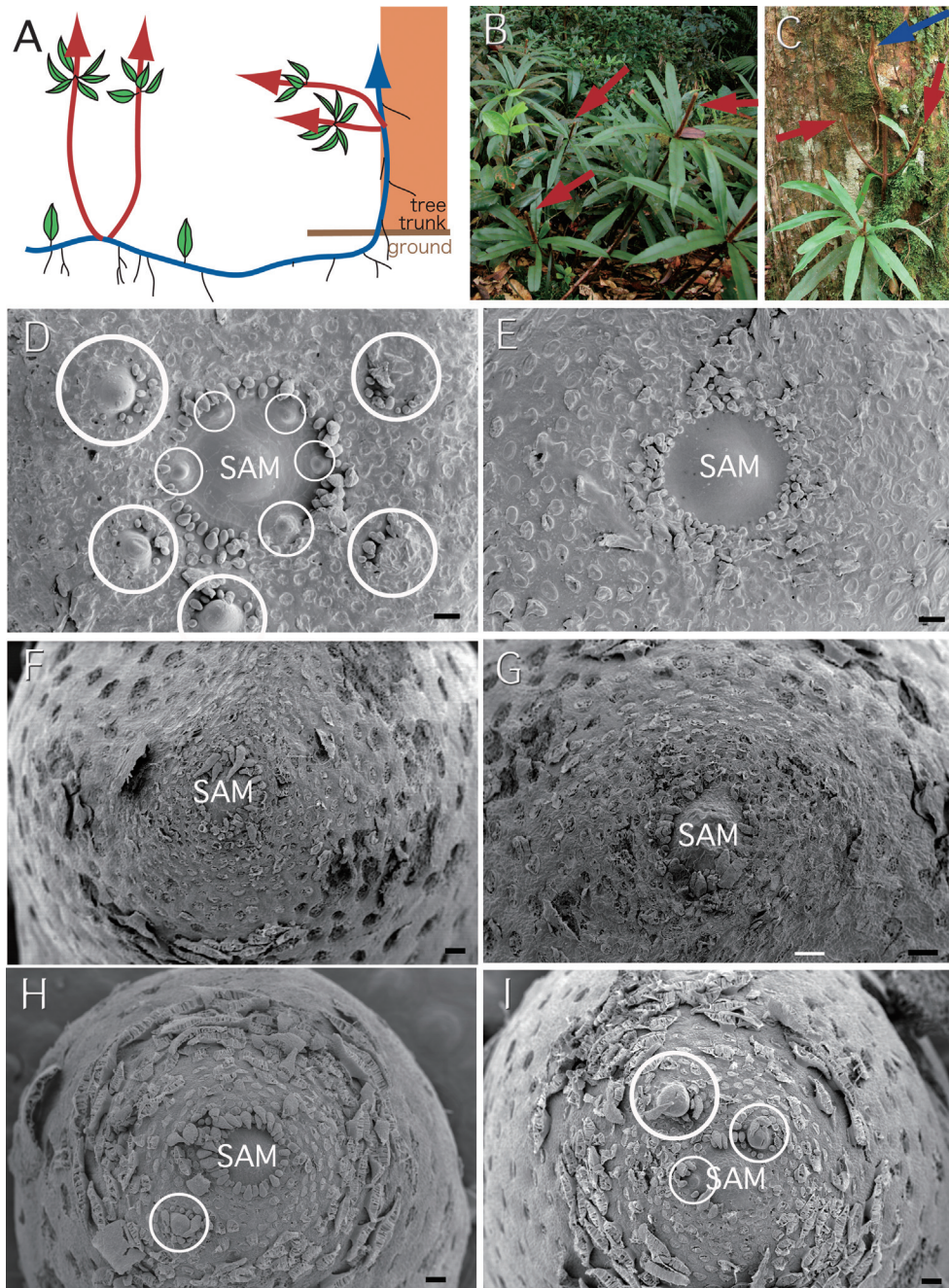


Fig. 1. Rhizomes of *Oleandra pistillaris*. A, Diagram showing rhizome dimorphism. Erect rhizome (red) with apparently whorled leaves and creeping rhizome (blue) with (a) few leaves. B, C, Plants in the field. Red arrows indicate erect rhizomes and blue arrow indicates creeping rhizome. D–I, SEM images of rhizome apices. White circles enclose leaf primordia. SAMs indicate shoot apical meristems. D, Erect rhizome with apparently whorled leaf primordia. E, Erect rhizome without leaf primordium immediately after branching. F, G, Creeping rhizomes without leaf primordium. H, Creeping rhizome with one leaf primordium. I, Creeping rhizome with three leaf primordia. Scales=100  $\mu\text{m}$ .



branching from the creeping rhizome, while the creeping rhizomes produced a few leaves, if any, at indeterminate sites. It is in contrast to the rhizomes of Davalliaceae and related ferns with a regular phyllotaxy (Croxdale, 1976; Hirsch and Kaplan, 1974; Wardlow, 1943). Another secondary hemiepiphytic *Nephrolepis* had also dimorphic rhizomes with leafy, short, erect shoots on the long stolon. The long stolon lost leaves in *N. cordifolia*. McAlpin and White (1974) reported that the stolon of *N. multiflora* does not produce leaf primordia until a new plant is organized after a prolonged period of growth. The rhizome habit is distinct from the monopodial rhizome of climbers and obligate epiphytes, rhizome elongation and leaf formation are sylleptic. Considering that the two secondary hemiepiphytes evolved independently (Tsutsumi and Kato, 2006), the dimorphic rhizomes were in association with secondary hemiepiphyte and not with phylogeny. The assumption of the association will be made robust by additional examples.

Some trees and twining or root-climbing liana produce two distinct types of shoots; short leafy shoots and long leafless shoots (e.g., Caesar and MacDonald, 1984; Critchfield, 1960, 1972; Hallé *et al.*, 1978; Ichihashi *et al.*, 2009; Maillete, 1982). A comparative study of nine lianas and two trees in Japan suggested that long shoots (treated as searcher shoots in the lianas) were much longer in stem-twining species and less leafy in most lianas than in trees, irrespective of the similarity of short shoots (treated as ordinary shoots in the lianas) between trees and lianas in several respects (Ichihashi *et al.*, 2009). Furthermore, *Marsdenia laxiflora* (Asclepiadaceae), a tropical twining liana, has both short leafy shoots and long twining shoots with no functional leaves (Peñalosa, 1982). Lianas require new physical supports to grow further, and the availability of suitable supports is critical for growth (Putz, 1984). The long shoot may be favored in stem-twining lianas, because the probability of finding a suitable support may increase with shoot length (Ichihashi *et al.*, 2009). In the secondary hemiepiphytic *Oleandra pistillar* and

*Nephrolepis* with rhizomes (stolons) climbing trees, the long creeping rhizome (stolon) and the erect rhizome producing leaves seem correspond to the long leafless shoots and the leafy short shoots of those lianas, respectively. Further studies are necessary to clarify the functions and ecological adaptations of the dimorphic rhizomes of the secondary hemiepiphytes.

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