

# A Preliminary Study on Mild Hemiparasitic Epiphytic Fern *Pyrrosia piloselloides* (Polypodiaceae)

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**Abstract** *Pyrrosia piloselloides* (L.) M.G.Price is an epiphyte preferring twigs of trees and is apt to have the twigs dried. We performed experimental observations of the epiphyte under the conditions of removal of the epiphyte and root anatomical observations of the epiphyte. The removal of the epiphyte progressed growth of the host tree. The root hairs of *P. piloselloides* were embedded 2 or 3 cell-layers within the cortex of the host branches. The preliminary conclusion is that *P. piloselloides* is a facultative mild-hemiparasitic epiphyte of fern.

**Key words** : epiphyte, hemiparasite, *Pyrrosia piloselloides*, root hair.

## Introduction

Epiphytic form is one of major life forms of plants, e.g. accounting for 9% of vascular plants and 25% of ferns and lycophytes (Zotz, 2013). Systematically, epiphytes are distributed in a wide spectrum of vascular plants, suggesting recurrent origins of epiphytism. Because of life on trees, epiphytes have a limited access to above-ground water and nutrient supplies, and are more diverse in the water economy and patterns of metabolism than any other rain forest synusia (Richards, 1996).

Host–epiphyte interactions have been widely documented. Epiphytes are considered not to establish metabolic relationships with host plants (Lüttge, 1989). Instead, they obtain water and nutrients from air and abiotic substrate material through several morphological and physiological adaptations (Benzing, 1990, 2000; Laube and Zotz, 2003; Zotz, 2016). Some epiphytes in several angiosperm families and ferns have adverse

effects in the host trees, such as defoliation and branch-death (Ruinen, 1953; Johansson, 1974; Benzing and Seemann, 1978; Montaña *et al.*, 1997; Soria *et al.*, 2014; Flores-Palacios, 2016). Negative effect of the epiphytes on the host trees has sometimes been treated as “epiphytosis” (Ruinen, 1953), “structural parasitism” (Stevens, 1987; Montaña *et al.*, 1997) or “piracy” (Benzing and Seemann, 1978). Mechanical damages, competition for light and nutrients, secondary effects, such as an increased susceptibility to pathogen attack and any allelopathic activity of some epiphytes, are proposed to cause such adverse effects (Benzing and Seemann, 1978; Caldiz and Fernández, 1995). Zotz (2016) noted that the nature of the impact of epiphytes on their host tree is not entirely understood. In some epiphytic orchids, bromeliads and ferns, root hairs were reported to penetrate living host stem-tissues (Furman, 1959), but to our knowledge, there is no clear evidence of the root penetration into host tissues.

The genus *Pyrrosia* belongs to Polypodiaceae, the largest epiphytic fern family, and comprises 50 epiphytic species (Hovenkamp, 1986). *Pyrrosia piloselloides* (L.) M.G.Price is an epiphyte usually growing on twigs of trees (twig epiphyte). The species causes defoliation and death of host tree-branches (Ruinen, 1953). Ruinen (1953) considered that the decline of host plant growth is caused by mycorrhizal fungi of epiphytes, which act as pathogen for host plants. Nonetheless, it may be possible that the epiphytic fern is a hemiparasite, although neither holoparasite nor hemiparasite is known in epiphytic ferns (Poulin *et al.*, 2000).

We performed field observation of the epiphytic *Pyrrosia piloselloides* and preliminarily investigated if the roots of *P. piloselloides* penetrate into host plants or not.

### Material and Methods

We made field observations of *Pyrrosia piloselloides* in the Bogor Botanic Gardens, Bogor, Indonesia. To examine the rates of rhizome elongation in the epiphytic *P. piloselloides*, we measured the length of the rhizomes on the host tree *Livistona chinensis* R.Br. (Palmaceae) every 2–3 weeks for 7 weeks from 14 July 2014 to 1 September 2014. To investigate the growth rates of host trees and the epiphytic *P. piloselloides*, we selected 6 trees of *Codiaeum variegatum* (L.) Rumph. ex A.Juss. (Euphorbiaceae) with *P. piloselloides*. All the trees had dense leaves and appeared healthy. The tree is evergreen and the measurements were performed in the rainy season. We selected two branches covered by *P. piloselloides* in each host tree under the treatments of (A) control (no treatment) and (B) removal of the epiphyte from the branch. The two branches were similar in the branch length, the leaf numbers, the coverage of *Pyrrosia*, and the light conditions. The branches were labeled on 2 or 3 December 2011, and we measured leaf numbers and lengths of the main branches of the host plants between the sites of labelling and the distal end. Then, we counted the numbers of

leaves and branch lengths of the host tree on 12 or 13 February 2013. We calculated the ratios of increase of leaf numbers and branch lengths in each branch.

For anatomical study, plants of *P. piloselloides* on host twigs were collected in October 2008. Host twigs with adhering roots of *P. piloselloides* were trimmed to about 3 × 3 mm blocks. The blocks were dehydrated in an ethyl alcohol series for more than 2 h and embedded in HistoResin Plus for 4 d (Leica, Heidelberg, Germany). The embedded materials were cut into 2- $\mu$ m thick sections with a glass knife on an ultramicrotome (LEICA RM2155, Leica, Vienna, Austria). Sections were stained with safranin, toluidine blue O, and orange G (Jernstedt *et al.*, 1992).

### Results and Discussion

Plants of *Pyrrosia piloselloides* were densely attached on twigs, branches, and trunks of several host species (Fig. 1). Twigs infested by the epiphyte were apt to dry, whereas infested thick branches and trunks were not apparently affected. We also saw many dried fallen twigs and thin branches densely covered by the epiphyte.

The rhizomes of *P. piloselloides* on *Livistona chinensis* grew nearly 3.3 mm long per day, although the elongation rates were various with different trees (Table 1). The rhizome with the highest elongation rate grew 454 mm long for 7 weeks. Bogor in West Java is tropical with the mean temperature of 25.2°C and the annual precipitation of 4,086 mm. It is semi-everwet with the dry season from June to September, in which the minimum monthly precipitation is 216 mm in July. The extensive growth of *P. piloselloides* in the dry season indicates that the plant is possible to cover the host branches in a few months even in dry season.

The relative increase of leaf number 14 months after the beginning of observation was higher in the host branches of *Codiaeum variegatum* with removal of the epiphytic *P. piloselloides* from the host tree than those with no



Fig. 1. Epiphytic *Pyrrosia piloselloides* on host plants. A. tree densely covered by the epiphyte. B. rhizome and roots of *Pyrrosia piloselloides* on a twig. C. rhizome and roots of *Pyrrosia piloselloides* on a host leaf. D. dried twigs fallen on the ground.

Table 1. Elongation of rhizomes of *Pyrrosia piloselloides* ( $N=6$ )

Time <sup>1</sup>	Increase of length of rhizome <sup>2</sup>
10 days	36.4 ± 35.9 mm
30 days	111.1 ± 106.3 mm
49 days	163.3 ± 150.4 mm

<sup>1</sup> Days after the beginning of observation.

<sup>2</sup> Mean ± S.D.

experimental treatment (Table 2). In comparison, 3 branches in control increased leaf foliation and 2 branches decreased. The removal of *P. piloselloides* resulted in a greater leaf production of the host than the control. The ratio of branch elongation during the term was also high in the branches with removal of the epiphyte, indicating that removal of the epiphyte resulted in a greater elongation of the host branch than control (Table 2).

Some epiphytes, such as *Tillandsia*, some

Table 2. Relative increase after 14 months (mean ± S.D.;  $N=6$ ) of leaf number and twig length in host tree *Codiaeum variegatum*

	Number of Leaf	Length of twig
Control	1.16 ± 0.46	1.25 ± 0.10
Removal of <i>P. piloselloides</i>	1.72 ± 0.46	1.47 ± 0.26

ferns and orchids, give disadvantageous effects to host trees (Ruinen, 1953; Johansson, 1974; Benzinger and Seemann, 1978; Montaña *et al.*, 1997; Soria *et al.*, 2014; Flores-Palacios, 2016). Like these previous studies, we found that the removal of the epiphyte resulted in the activated growth of the host than in control, and suggested the disadvantageous effect by the epiphytic *P. piloselloides* to the host. However, as stressed by Ruinen (1953), the effects of the epiphytes may not always be strong and rapid. Although host twigs were sometimes dried in field observations,

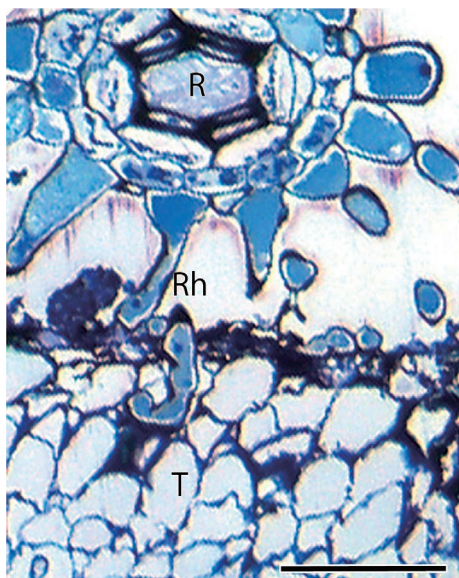


Fig. 2. Section of root of *Pyrrosia piloselloides* on a host twig. R=root. Rh=root hair. T=host twig. Scale bar = 100  $\mu$ m.

we could not encounter dried twigs in this experimental investigation.

In anatomy, the root hairs of the epiphytic *P. piloselloides* on the ventral side of root were embedded 2 or 3 cell-layers within the cortex of the host branches (Fig. 2). Penetration of root hairs of the epiphyte into the host tissue were low-frequently observed. It suggests that the epiphyte has an ability to absorb water and nutrients from host tissues, in addition to use of external ones. However, the root hairs neither connect with the vascular tissues of host plants, nor the roots are specialized like haustoria, and therefore, differ from the roots of parasitic plants (Kuijt, 1969; Heide-Jørgensen, 2008). *Pyrrosia piloselloides* is not a typical parasite but can be a facultative mild hemiparasite. In our field observation, roots of some plants of Araceae penetrated into host trees (data not shown). It is possible that some epiphytes absorb water and nutrients by penetrating the roots into host trees and are facultative mild hemiparasites. Parasites represent highly specialized groups that are systematically and physiologically distinct from epiphytes, i.e., Apodanthaceae, *Cassytha* (Laurales),

*Cuscuta* (Convolvulaceae), Cynomoriaceae, Cytinaceae, Hydnoraceae, Krameriaceae, Lennoaceae, Mitrastemonaceae, Orobanchaceae, Rafflesiaceae, Santalales with Balanophoraceae (Madison, 1977; Barkman, *et al.*, 2007). Our preliminary study suggests that epiphytes may be able to evolve further to a mild-hemiparasitic lifestyle. Further studies are necessary to examine other epiphytes and clarify the transportation of water and nutrients between epiphytes and host plants.

The mechanism by which the host twigs infected by the mild hemiepiphytic *P. piloselloides* dry up is uncertain. The decrease of host growth may be due to, e.g., mechanical damages, competition for light and nutrients, an increased susceptibility to pathogen attack and any allelopathic activity of some epiphytes (Benzing and Seemann, 1978; Caldiz and Fernández, 1995). Ruinen (1953) considered the main cause of the adverse effect to host plant growth is a mycorrhizal infection through epiphytic roots. Furthermore, parasitism generally reduces host growth (see review in Press and Phoenix, 2005), and in the most extreme cases may result in host death (Aukema, 2003). To clarify the decrease of host-plant growth by *P. piloselloides*, further studies are necessary.

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