



# Zeylanidium as Pollution Indicators: Live Sensors to Water Parameters

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## Abstract

Podostemaceae family is well known as a group of aquatic angiosperms with enigmatic characters. The present work was performed to trace ecological aspects, phenology, water analysis and phylogenetic analysis of the genus *Zeylanidium*, in order to establish the uniqueness of the species in the fresh water habitats S-1 (9.9559°N, 76.8339°E) and S-2 (10.0538°N, 76.8294°E). Through water analysis it is revealed that the species of plants could be used as an indicator of pollution as they are present in very sensitive area of running fresh water. The phenology of the taxa under study paved light to the co-existence of *Zeylanidium maheshwari* and *Zeylanidium lichenoides*. The data on comparison of molecular and phylogenetic tree reveal that the species are highly related genetically for a sustainable coexistence.

## Subject Areas

Plant Science

## Keywords

Zeylanidiumas

## 1. Introduction

Podostemaceae is the largest family of submerged flowering plants having unusual morphology and reproductive characters. Recent results based on molecular analysis indicate that Podostemaceae belongs to the Malpighiales clade, most closely related to Clusiaceae and Hypericaceae. Tristichoideae and Weddelli-noideae are acceptable as podostemaceous subfamilies, which are clearly distinguishable from the large subfamily Podostemoideae [1] [2].

Geetha [3] and Girija [4] studied the ecological aspects of Podostemaceae present in Kerala by assigning different sites of Kerala into highland, lowland and

midland based on topology and elevation from main sea level. They have studied the geology and rock specificity of the taxa. Greenand Gyure [5] characterized bacterial communities associated with members of the plant family Podostemaceae. *Cyanobacteria* (blue green bacteria) play a major role in the secretion of adhesive compounds that allow these plants to attach to rocks in waterfalls, and thus survive rapid flowing water and rapidly changing environmental conditions.

Most authors have classified the family into two subfamilies, Podostemoideae and Tristichoideae [6] [7] [8] [9] Engler [10] divided Podostemaceae into three subfamilies such as Podostemoideae (35 genera), Tristichoideae (three genera), and Weddellinoideae (monotypic). The molecular data support them as independent lineages [2] [11].

The Podostemaceae are strictly aquatic and tropical and are seen in unique habitat attached to rocks in river rapids and waterfalls. They have adopted many special anatomical and morphological characters [12] in order to counteract the destructive force of water. Earlier it was believed that they were attached to the rocks by means of a cementing material secreted from their haptera [13]. Later the studies conducted by the Jager-Zurm and Grubert [14] revealed that the strong attachment of the taxa to the substratum is via an extracellular polymeric substance of the bacterial biofilm present in their habitat. Moline *et al.* [11] studied the matK sequence data along with morphological data of eight African species of Podostemaceae belonging to the genera *Dicraeanthus*, *Dijnga* and *Ladermanniella*. Khanduri *et al.* [15] carried out a phylogenetic study of the Indian Podostemaceae using its sequence data and morphological characters. The phylogenetic analysis reveals that the Indian Podostemoideae is subdivided into two subclades; one represented by *Willisia* and *Podostemum* and the other by *Zeylanidium*, *Hydrobryopsis*, *Griffithella* and *Polypleurum*.

The present conducted during South-West monsoon 2013 through summer 2017 in order to focus on ecological aspects, phenology, water analysis and phylogenetic analysis of the podostemaceae genus *Zeylanidium*, and to establish the uniqueness of the species in the fresh water habitats.

## 2. Materials and Methods

Fresh water habitats S-1 (9.9559°N, 76.8339°E) and S-2 (10.0538°N, 76.8294°E) were selected after pilot study for presence of the selected study species. Clear water samples close to the species under study that extends 0.5 m to 1m vertical depth were obtained using insulated bottles and brought to the laboratory to study the parameters such as dissolved oxygen, free Carbon dioxide, alkalinity [16], nitrite determination and sulphate.

Soon after collection, plants were examined in order to establish correct identification and also referred with flora [17]. Technical terms for external appearance of the plant structures are given in order to explain the morphological features of the species under study and they are adopted from previous explana-

tions given by Girija [4], Mathew and Satheesh [18] and Nileena [19]. The characters and the character states considered for the present study is given in **Table 1**. The selected morphological characters were used to create a matrix and evolutionary tree were drawn using Mesquite's heuristic tree search and a consensus tree were generated by using Mesquite 2.75 [20]. In order to study the phylogeny of the family podostemaceae and to infer the systematic position of *Zeylanidium* the character optimization of morphological characters was examined by using Mesquite 2.75 [20] molecular systematic analysis was done by using the gene matK.

The matK genes of the different species of Podostemaceae along with 1 out group (clussiaceae) were retrieved from genbank of National centre for biotechnological information (<http://www.ncbi.nlm.nih.gov/genbank/>) (**Table 2**).

The sequences were aligned using ClustalX 2.0.11 [21]. The gaps and missing data were eliminated. Phylogenetic analyses were done using Bayesian (maximum posterior probability, MPP), maximum parsimony (MP), and neighbor-joining (NJ). Phylogenetic analyses by MP [22] [23] [24] and NJ [24] methods were conducted using MEGA ver. 6 [25].

### 3. Results and Discussions

S-1 a low-land area (9.9559°N, 76.8339°E) and S-2 a high-land area (10.0538°N, 76.8294°E), which experiences tropical climate and belongs to Idukki district. Hornblende-biotite gneiss rock is the major type of rock present in these sites. *Zeylanidium maheshwarii* and *Zeylanidium lichenoides* were collected along with *Polypleurum stylosum* in S-1. (S-2) is the midland area of Idukki district. Water sampling data of the two sites are given in **Table 3**. The site experiences subtropical climate and possess charnockite rocks. *Zeylanidium olivaceum* flourishes well here along with *Zeylanidium maheshwarii*.

Phenological patterns of podostemads under study are represented in phenograms. In Thommenkuth, *Zeylanidium maheshwarii* and *Zeylanidium lichenoides* can be seen. They grow intermingled and show a similar phenology. Hence the co-occurrence of both this taxa gives a strong relationship between them. According to Willis [17] the water level and position of taxon on the rocks control the phenological pattern of these taxa in a specific region. As the genus *Zeylanidium* resides on higher rock position this may be the reason why they show similar phenological pattern. The phenological pattern of *Zeylanidium olivaceum* is slightly different from **Figure 1**. Phenogram of two taxon. According to Girija [4] this difference stands even though they are seen along with other taxa (**Figure 2**).

The topology of maximum likelihood tree (ML), maximum parsimony tree (MP), and neighbour-joining tree (NJ), Mr bayes tree are similar indicating the reliability of the phylogenetic tree that have been drawn and presented in **Figure 3**. In the molecular phylogeny tree, two clades can be identified—1. Tristichioideae, 2. Podostemoideae. Hence the monophyly of each subfamily were

**Table 1.** Morphological characters and character states used in the study.

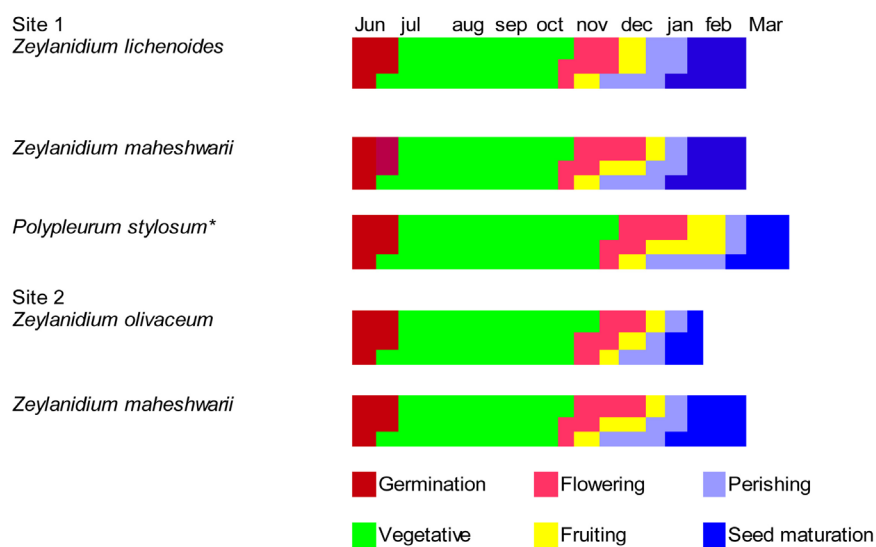
Character	Character states
shoots	0 = monomorphic, 1 = dimorphic.
Leaf base	0 = narrow attachment point, 1 = sheathing
stipules	0 = absent, 1 = present.
Leaf type	0 = simple, 1 = compound, 2 = ramulus.
Leaves	0 = monomorphic, 1 = dimorphic.
Vegetative stem length	0 = less than 1, 1 = 1 - 5 cm, 2 = greater than 5 cm.
shoot position	0 = only at sinus of thallus branching, 1 = on lateral flank along length of thallus including the sinus of thallus branching dorsal surface in foliose thallus.
Double sheathing leaf	0 = absent, 1 = present.
Maximum leaf length	0 = 1 cm, 1 = 1 - 5 cm, 2 = above 5.
spathella	0 = absent, 1 = present.
Spathella tip	0 = round, 1 = pointed, 2 = beaked.
Spatella surface	0 = 0 = smooth, 1 = hairy.
Spatella rupture	0 = longitudinal, 1 = irregularly at the apex.
Cupule	0 = absent, 1 = present.
symmetry of flower	0 = actinomorphic, 1 = zygomorphic.
Flower grouping	0 = solitary, 1 = cluster
flower pedicel	0 = absent, 1 = present
flower orientation at anthesis	0 = vertical, 1 = dorsiventral, 2 = oblique
floral bract	0 = absent, 1 = present.
Bract	0 = absent, 1 = simple, 2 = digitate.
Perianth	0 = absent, 1 = present
tepal	0 = six or more in complete whorl, 1 = 5 as complete 2 = 2, 3 = 3 as complete whorl.
Staminode	0 = absent, 1 = present
third staminode in anthropodium	0 = absent, 1 = present
andropodium	0 = absent, 1 = present
stamen whorls	1 complete whorl, 2 = additional whorl.
Number of stamens	0 = more than 3, 1 = 2, 2 = 3, 3 = 1.
pollen aperture	0 = pantoporate (polyporate), 1 = tricolporate, 2 = tricolpate
pollen	0 = monad, 1 = dyad.
Carpel number	0 = 2, 1 = 3
Symmetry of ovarian locules	0 = isolobulous, 1 = anisolobulous
locule number	0 = 2, 1 = 3, 2 = 1
stigma lobe	0 = 2, 1 = 3, 2 = multilobed.
Stigma form	0 = linear, 1 = fan shaped, 2 = capitate.
Stigma margin	0 = entire, 1 = irregular.
Stigma lobe	0 = equal, 1 = unequal
gynopore	0 = absent, 1 = present
Ovary position in spatella	0 = erect, 1 = oblique, 2 = inverted
capsule shape	0 = globose, 1 = elliptical, 2 = globular - ellipsoid
capsule morphology	0 = absent (smooth), 1 = present (ribbed).
Number of rib per valve	0 = nil, 1 = 3, 2 = more than 3
capsule valves	0 = persistent, 1 = deciduous

**Table 2.** The matK genes of different species of Podostemaceae.

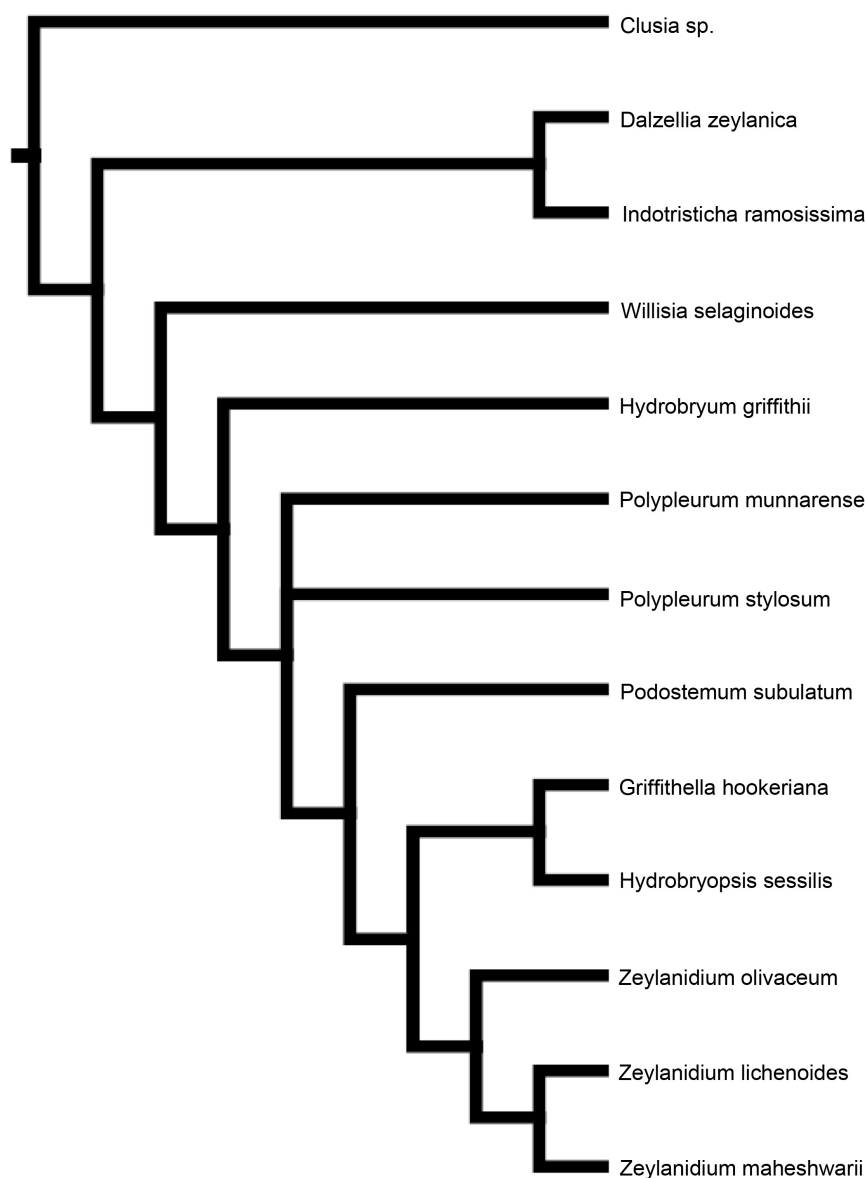
Taxon	Locality	
<i>Dalzellia zeylanica</i>	Sree Lanka	AB450023
<i>Farmeriametzgerioides</i>	Sree Lanka	AB698232
<i>Griffithella hookeriana</i>	Kerala	AB698236
<i>Hydrobryopsis sessilis</i>	Kerala	AB698240
<i>Hydrobryum griffithii</i>	Thailand	AB104570
<i>Podostemum subulatum</i>		AB038207
<i>Polypleurum munnarensense</i>	Kerala	AB610267
<i>Polypleurum stylosum</i>		AB066174
<i>Willisia selaginoides</i>		AB698236
<i>Zeylanidium lichenoides</i>	Kerala	AB048828
<i>Zeylanidium maheshwaraii</i>	Kerala	AB048379
<i>Zeylanidium olivaceum</i>	Sri Lanka	AB038207
<i>Clussia criuva</i>		AB450037

**Table 3.** Water sampling data.

Parameter	(S-1)	(S-2)
Dissolved oxygen	9.3 mg/l	10.73 mg/l
Free carbondioxide	6.16 mg/l	5.28 mg/l
Alkalinity	20 ppm	50 ppm
Nitrite	Nil	Nil
Sulphate	Nil	Nil

**Figure 1.** Phenological pattern of podostemaceae under study.

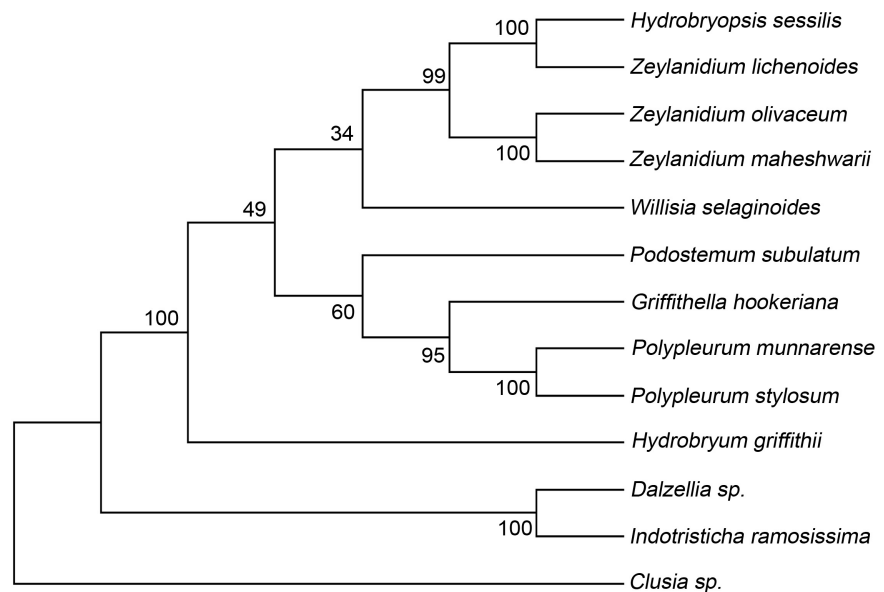
supported, in accordance with earlier studies [2] [11] [15]. The clade Tristichoideae includes *Dalzellia zeylanica* and *Indotristica ramossisima*. The Podostemoideae is divided into two sub-clades. One clade comprises of *Podostemum subulatum*,



**Figure 2.** Morphological tree of Indian Podostemaceae.

*Griffithella hookeriana*, *Polypleurum stylosum*, and *Polypleurum munnarensense* and the other includes *Willisia selaginoides*, *Hydrobryopsis sessilis*, *Zeylanidium lichenoides*, *Zeylanidium maheshwarii* and *Zeylanidium olivaceum*. This is in agreement with the study of Khanduri *et al.* [15], which showed that *Willisia* and *Podostemum* form a clade and *Polypleurum*, *Hydrobryopsis*, *Griffithella* and *Zeylanidium* forms another clade.

The analysis of morphological characters yielded a consensus tree. It has much similarity with the tree drawn by molecular phylogeny. It also yielded two clades—Tristichoidae and Podostemoidae. The difference with the molecular phylogenetic tree is in the positioning of *Griffithella hookeriana*. Here *Zeylanidium maheshwarii* and *Zeylanidium lichenoides* becomes a clade while in the molecular phylogenetic tree *Zeylanidium maheshwarii* and *Zeylanidium olivaceum*



**Figure 3.** Maximum parsimony tree of Indian Podostemaceae.

becomes a clade. In both the phylogenetic trees the genus *Zeylanidium* is closely related to *Hydrobryopsis sessilis*. Cusset and Cusset [1] proposed that the Indian species of *Podostemum* should be shifted to the genus *Zeylanidium*. However the present morphological and phylogenetic analysis suggests that the Indian species of *Podostemum* should not be included in the genus *Zeylanidium*.

#### 4. Conclusion

*Zeylanidium lichenoides* is a widely distributed genus and maximum distribution of this taxon indicates its primitiveness in the lowland. *Zeylanidium maheshwarii* is a highly restricted species growing well on the dorsal surface of rocks. The taxa show a close association with *Zeylanidium lichenoides*. There occurs a marked similarity between the taxa in terms of phenology. This along with the co-occurrence of the taxa indicates the closeness of the taxa. Co-occurrence is an interesting phenomenon and important from the evolutionary point of view. Sometimes co-occurrence may result in hybridization and probably through this incidence, self-fertilized and homozygous plants will show variability in their genome. Eventually they will be able to survive, compete and show wider distribution. Thus *Zeylanidium lichenoides* has followed this strategy and *Zeylanidium maheshwarii* appears to have evolved as a new species from this through a slight change in the environmental conditions, water regime and water chemistry. The co-occurrence of *Zeylanidium maheshwarii* with *Zeylanidium lichenoides* benefits the taxon by giving protection, animal visits and also ensure continuous water supply. Considering all these, *Zeylanidium lichenoides* can be considered as the primitive one. *Zeylanidium maheshwarii* has got an advanced position with specific requirements. The sister group concept of this taxa generated by mat k gene phylogenetic analysis gives additional support

to this family. The phenology of the taxa under study paved light to the co-existence of *Zeylanidium maheshwari* and *Zeylanidium lichenoides*. The data on comparison of molecular and phylogenetic tree reveal that the species are highly related genetically for a sustainable coexistence. Identification of plants with such characters can be high significance in future habitat and species conservation programs. The family Podostemaceae is seen in the water falls of Western Ghats regions indicating its need of specific substratum for growth. Also earlier studies indicate their close association with Cyanobacteria. Such associations might have some direct relationship with the existence and life cycle pattern of Podostemaceae, which has to be resolved by further studies.

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