



Epiphyllous algae of *Trentepohlia* Martius in Southwestern Ghats, India, including one new species and three new records

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ABSTRACT: Altogether eight species of epiphyllous green algae, *Trentepohlia* were discovered from the rural and semi-urban areas of Southwestern Ghats by field observations and *in-vitro* culture investigations. The species found were *T. abietina* (Flotow & Kutzing) Hansgirg, *T. rigidula* (J. Muller) Hariot, *T. annulata*, F. Brand, *T. aurea* (L.) Martius, *T. chapmanii* Rindi & Lopez-Bautista, *T. dialepta* (Nylander) Hariot, *T. arborum* (C. Agardh) Hariot, and *T. gaviensis* Binoy T.T., Bhagya M.V. & V.P. Thomas *sp. nov.* They were isolated and observed from the leaves of fourteen host plants. All the species were seen in the areas exposed to full sunlight. *Trentepohlia rigidula* and *Trentepohlia abietina* were also observed from the *in vitro* culture in Bolds Basal Medium (BBM). Three of the species, *T. abietina*, *T. rigidula*, and *T. chapmanii* and were the new records from Kerala; whereas *T. dialepta*, *T. annulata*, *T. aurea*, and *T. arborum* have been reported in earlier studies. A new species of *Trentepohlia* is compared with its closely related *T. sundarbanensis* (G.G. Satpati & R. Pal), *T. arborum* (C.Ag.) Hariot and *T. abietina* (Flotow & Kutzing) Hansgirg and is also described in the present study. So far, the diversity of *Trentepohlia* in tropical regions, particularly in rural and semi-urban areas of Kerala was quite unknown. The present investigation indicated that the Southwestern Ghats region of Kerala represents a major habitat zone of epiphyllous algae, particularly *Trentepohlia* which demands further extensive investigations in the area to explore the complete report of the entire diversity of algae.

KEY WORDS: Epiphyllous algae, India, Semi-urban areas, Terrestrial algae, *Trentepohlia*, Trentepohliaceae, Western Ghats.

INTRODUCTION

Phyllosphere of plants contains characteristic and diverse assemblages of coccoid green algae and cyanobacteria (Ettl and Gartner, 1995; Hoffman, 1989; Neustupa and Skaloud, 2008). The epiphytic macroscopic algae are dominated by Trentepohliales (Brand and Stockmayer, 1925) consisting of *Trentepohlia* Martius. The Trentepohliales are widely distributed sub-aerial green algae growing on wood, tree bark, leaves, rock, building walls and several other types of artificial substrata (Printz, 1939; Rindi and Lopez-Bautista, 2008; Rindi *et al.*, 2006; Chapman, 1984; Ettl and Gartner, 1995) which are exposed to full sunlight (Chua *et al.*, 1972; Binoy *et al.*, 2017). It is widespread in the regions with humid climates; diverse in the tropics; however, it is also present in temperate regions (Printz, 1939; Rindi *et al.*, 2004). *Trentepohlia* belongs to the order Trentepohliales under the division Chlorophyta (Rindi and Lopez-Bautista, 2008; 2007). It is the largest genus in the order, which consists of 6 genera and 35 species which belong to the single family, Trentepohliaceae (Printz, 1939; Thomson and Wujek, 1997). The genus is recognizable by the presence of orange-red colour (Ong *et al.*, 1992) due to the presence of β -carotene and haematochrome. It has been studied intensively; however, the exact diversity of the genus from tropics is still limited. The shape, size of vegetative cells, the presence of setae, branching pattern and morphology of reproductive structures (Printz, 1939) are usually considered for the identification of

Trentepohlia at the species level. However, owing to remarkable fluctuation in the environmental conditions, several morphological changes have been reported (Chapman and Henk, 1985; Roberts, 1984). *Trentepohlia* differs from other green algae by the lack of pyrenoids in the chloroplast (Rindi *et al.*, 2009). *Trentepohlia* is commercially precious due to the lipids and pigments in them (Hirano *et al.*, 1995; Hirano *et al.*, 1999; Mendoza *et al.*, 1996; Qiang *et al.*, 1997; Qiang *et al.*, 1998). They have high tolerance and adaptability to tide over desiccation at high temperature; so that the algae can develop better defense systems against photooxidative damage (Ong *et al.*, 1992). These species also are known to have the ability to accumulate large quantities of β -carotene, vitamin E and vitamin C in their cells (Hirano *et al.*, 1999). The present study is aimed at exploring the diversity and taxonomy of the *Trentepohlia* species from the phyllosphere of Southwestern Ghats regions, Kerala, India.

MATERIALS AND METHODS

Sampling

Algae associated leaves were collected from six different sites (each was of about 1–2 acre land) of Pathanamthitta District (9.2601°N, 76.9643°E), Kerala. Among the sites, Adoor, Thiruvalla, and Kaipattoor are semi-urban areas and Konni, Gavi and Ranni are rural areas with natural forest (Fig. 1). Samples were collected from September 2016 to September 2017. Voucher



specimens of *Trentepohlia* spp. were kept in the Catholicate Phycotechnology Herbarium (CAPH) and CATH Herbarium, Catholicate College, Pathanamthitta, Kerala, India with accession numbers.

Isolation, cultures, and microphotography

The epiphyllous algae were isolated from the collected leaves by brush and scalpel. The specimens were examined either directly from the field or from the uni algal culture samples. The isolated *Trentepohlia* species were cultured in the Modified Bold's Basal Medium (MBBM). Triplicate samples of algae were observed from each host plant. Microscopic features were examined by using the Olympus LX 400 Trinocular microscope and photographs were taken by using BioLinkz Cmos Cam (3.0m pixels) attached to the microscope. Identification was based on the monograph, database and the systematic keys (Thompson and Wujek, 1997; Guiry and Guiry, 2016; Printz, 1939).

Diversity Analysis

Diversity index of the algae was analysed by Paleontological Statistics software package for education (PAST 2.17c)

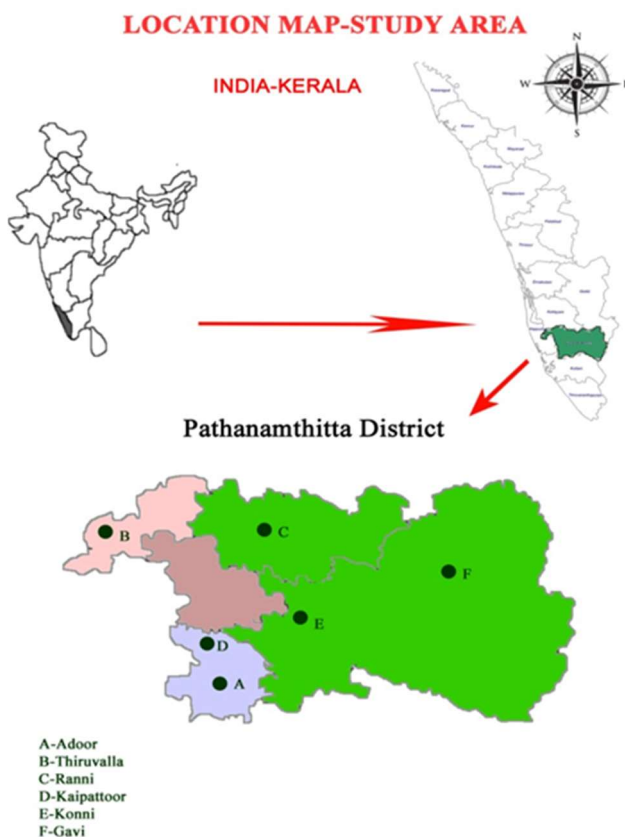


Fig. 1. Map of Pathanamthitta district, Kerala, India

TAXONOMIC TREATMENTS

The present investigation explored eight species of *Trentepohlia* belong to the class Trentepohliaceae from the fourteen host plants. The semi-urban area had a higher number (7 species) of *Trentepohlia* than the rural area (5 species). Their diversity index (1.55) was also higher in the semi-urban area than the rural area (1.33).

1. *Trentepohlia abietina* (Flotow & Kutzing) Hansgirg, Prodrum der Algenflora von Bohmen. 1886, 5 (6): 86, Basionym- *Chroolepus abietinus* Flotow ex Kutzing, Phycologia Germanica 1845: 228.

Fig. 2A

Description: Erect axes of the thallus arising from a limited prostrate system which is either little or not branched. The prostrate system has globular or elliptical cells with 6–9 μm in diameter and 18–20 μm in length. Cells of the erect axes are cylindrical or barrel-shaped. Apical cells are usually larger, often bearing an apical cap. Septa between adjacent cells are not noticeably thicker than lateral walls.

Notes: New record in Kerala; earlier reported at Shillong from the bark of trees (Kharkongor and Ramanujam, 2015); golden orange thallus of the alga appeared on the leaves and twigs. It was found in all seasons, particularly during northeast monsoon season. It was developed in Bolds Basal Medium.

Specimens examined: INDIA, Kerala, Pathanamthitta District, Thiruvalla & Kaipattoor, semi-urban area. Alga forming mats on twigs and leaves of *Xanthophyllum flavescens* Roxb. (Family-Polygalaceae, CATH-12026); *Artocarpus integrifolia* L. (Family-Moraceae, CATH-12006); *Vateria indica* L. (Family-Dipterocarpaceae, CATH-12058); *Aporosa cardiosperma* (Gaertn.) Merr. (Family-Euphorbiaceae, CATH-12041); *Memecylon umbellatum* Burm.f. (Family-Melastomaceae, CATH-12048) 28 September 2017, Binoy TT, Thomas VP & Bhagya M V, CAPH-130.

2. *Trentepohlia rigidula* (J. Muller) Hariot. Journal de Botanique 1889 3: 403, Basionym: *Coenogonium rigidulum* J Muller. Lichenologische Beitrage. 65(31): 483-490. 1882.

Fig. 2B

Description: The erect filaments are profusely branched and the apex is not tapered. Vegetative cells are elliptical, barrel-shaped with 20–25 μm wide and 45–60 μm long. In between the two adjacent cells, a septum or strong constriction is formed. Sporangium varied from globular or dome-shaped and formed on the filament with apical, intercalary or in the lateral position. Sporangia of *T. rigidula* are globose, sometimes oval and larger than the vegetative cells.

Notes: New record in Kerala; earlier examined from West Bengal (Satpati and Pal, 2016); entangled filaments with cottony mass was generally seen on the leaves in all seasons, prominent during summer and southwest monsoon season. It was completely absent in the shaded region and developed in the Bolds Basal Medium.

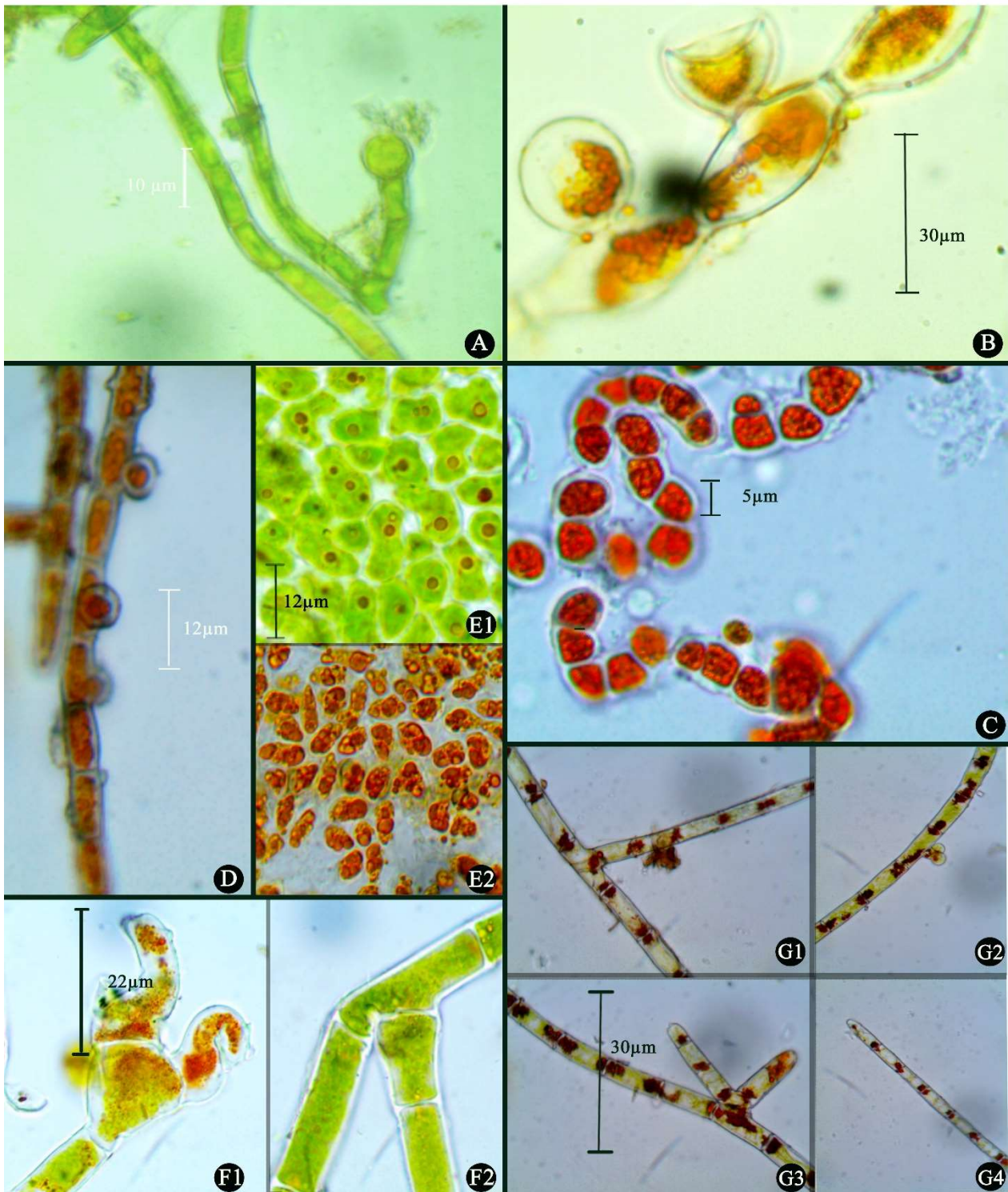


Fig. 2. Epiphyllous algae of *Trentepohlia* Martius in Southwestern Ghats, India. **A.** *Trentepohlia abietina*. **B.** *Trentepohlia rigidula*. **C.** *Trentepohlia annulata*. **D.** *Trentepohlia aurea*. **E.** *Trentepohlia chapmanii*. **F.** *Trentepohlia dialepta*; **G.** *Trentepohlia arborum*.

Specimens examined: INDIA, Kerala, Pathanamthitta District, Adoor, semi-urban area, *Trentepohlia rigidula* found on the leaf surfaces of *Mangifera indica* L. (Family-Anacardiaceae, CATH-

12039); *Drynaria quercifolia* (L) J. Sm; Hook (Family-Polypodiaceae, CATH-12017); *Hydnocarpus wightianus* Blume (Family-Achariaceae CATH-12071), 22 May 2017, Binoy T T & Bhagya M V, CAPH-131.



3. *Trentepohlia annulata* F. Brand. Beihefte zum Botanischen Centralblatt 12(2): 222, 1902.

Fig. 2C

Description: Thallus is formed by erect axes and without branched, arising from the prostrate axes which are poorly developed. Certain new branches are raised from the centre of the prostrate cells. Cells are cylindrical with 18–27 μm length and width range from 7–10 μm . Cells are 2–3 times as long as wide. Cross walls between the two adjacent cells are thicker than the lateral walls.

Notes- Earlier it was reported at Kerala from the bark (Bast *et al.*, 2015), appeared as dark red patches on the leaves of specific host plants; thallus can be seen by the naked eye; found in all seasons, particularly during the summer season. It was absent in the shaded region.

Specimens examined: INDIA, Kerala, Pathanamthitta District, Adoor, semi-urban area. Alga found on leaf surfaces of *Myristica malabarica* Lam. (Family-Myristicaceae, CATH-12081) 12 March 2017, Binoy T T & Bhagya M V, CAPH-134.

4. *Trentepohlia aurea* (L.) Martius Flora cryptogamica 1817: 351; **Basionym:** *Byssus aurea* L. Species Plantarum 1753: 1168. 1753.

Fig. 2D

Description: The regularly branched erect axes are developed from a limited prostrate system. Filaments are ornately branched, erect, not tapered to the apex, not constricted and devoid of pectic caps. The cells of the main axis are cylindrical, but lateral branches have either cylindrical or barrel-shaped cells. New branches are born from the central part of the filaments. Cell length ranges from 2–4 μm and width ranges from 8–12 μm . Gametangia are not frequently observed.

Notes: In the earlier record it was examined in Kerala (Panikkar and Sindhu, 1993). However, in the current study, it was appeared as dense green-orange cushions on the leaves from the rural area, macroscopically visible, found in all seasons, prominent during the north east monsoon seasons.

Specimens examined: INDIA, Kerala, Pathanamthitta District, Gavi and Ranni, rural areas. Epiphytically seen on the leaves of *Helicteres isora* L. (Family-Malvaceae, CATH-12039), 9 December 2016, Binoy T T & Thomas V P, CAPH-135.

5. *Trentepohlia chapmanii* Rindi & Lopez-Bautista. Phycologia 46:699, 2007

Fig. 2E

Description: The prostrate part composed of many filaments, densely entangled and irregularly branched. No distinct individual filaments are recognized. Occasionally, numerous, thin and cylindrical erect axes are differentiated from the prostrate part. Cells are usually polygonal and 16–25 μm wide.

Notes: New record in India; morphologically the thick pseudoparenchymatous compact thallus is similar to the genus *Phycopeltis*. It was found in all seasons,

particularly during north east monsoon seasons.

Specimens examined: INDIA, Kerala, Pathanamthitta District, Thiruvalla, semi-urban area, and Ranni, rural area. Alga is common to the host plants of *Vateria indica* L. (Family-Dipterocarpaceae, CATH-12058), *Xanthophyllum flavescens* Roxb. (Family-Polygalaceae, CATH-12065), *Madhuca nerifolia* (Moon) H.J. Lam (Family-Sapotaceae, CATH-12051), and *Clusia species* (Family-Clusiaceae), 15 September 2016, Binoy T T, Thomas VP & Bhagya M V, CAPH-133.

6. *Trentepohlia dialepta* (Nylander) Hariot. Journal de Botanique 3: 386, 1889; **Basionym:** *Coenogonium dialeptum* Nylander Annales des Sciences Naturelles 1862 416: 90.

Figs. 2F

Description: The species appears as yellowish green tufts of filaments with distinct prostrate and erect branches tapering towards the end. The cell is mostly cylindrical, 13–15 μm wide and 29–50 μm long. Gametangia are spherical, lateral, solitary and 18–24 μm in diameter. Sporangia are more or less ovoid, not more than 19 μm wide and 16–19 μm long. Stalk cells are bottle-shaped, often bent at neck, not more than 26 μm wide and 13–14 μm long.

Notes: New record in Kerala (Binoy *et al.*, 2017); alga was found in all seasons on the surfaces of specific host plants; prominent during the summer season; a tuft of filaments seen at the open canopy region.

Specimens examined: INDIA, Kerala, Pathanamthitta District, natural forest in Ranni forest division, rural area, Epiphyllous on the leaves of *Madhuca nerifolia* (Moon) H.J. Lam (Family-Sapotaceae, CATH-12051) 18 April 2017, Binoy T T & Thomas VP, CAPH-132.

7. *Trentepohlia arborum* (C. Agardh) Hariot Journal de Botanique 3:383. 1889. **Basionym-** *Conferva arborum* C. Agardh; Systema Algarum 1824: 88.

Fig. 2G

Description: The thallus is entangled by branched filaments. The cells are cylindrical, 45–62 μm long, 17–22 μm wide; length being 2–4 times wide. The shapes of the apical cells are varied; erect and tapered to the apex, devoid of pectic caps and not constricted. The branches are irregular and longitudinal to each other. Grouped sporangia from a basal swelled cell or suffultory cell are the unique characteristic feature.

Notes: Earlier reported from Kerala and Shillong from rocks (Panikkar and Sindhu, 1993; Kharkongor and Ramanujam, 2015). Alga appeared on the margin of the leaves as green-orange tufts of cottony mass. It was found in all seasons, particularly during the summer season.

Specimens examined: INDIA, Kerala, Pathanamthitta District, Konni, rural area, Adoor & Kaipattoor, semi-urban area. Alga was found on the leaf surfaces of *Artocarpus terophyllus* L. (Family-Moraceae, CATH-12064) and *Artocarpus integrifolia* L. (Family-Moraceae, CATH-12006) 18 April 2017, Binoy T T, Thomas VP & Bhagya M V, CAPH-136.



8. *Trentepohlia gaviensis* T.T. Binoy, M.V. Bhagya & V.P. Thomas, *sp.nov.*

Fig. 2A-H

Type: INDIA, Kerala, Pathanamthitta District, Gavi, natural forest, 77.1658°E, 9.4359°N Epiphyllous on *Pavetta indica* L. (Rubiaceae). 9 December 2016, Binoy T T & Thomas VP 141 (Holotype CAPH!, Isotype CATH!).

Diagnosis: *Trentepohlia gaviensis* is closely similar to *Trentepohlia sundarbanensis*, Phykos, G.G. Satpati & R. Pal, 2015:45. Other related species are *T. arborum* (C. Ag.) Hariot and *T. abietina* (Flotow & Kützing) Hansgirg. The distinct features are heterotrichous plant body; rudimentary prostrate axes; profuse and regular branching in the main axis, which are mostly opposite; rarely alternate. These laterals are arranged in monopodial fashion. Each cell is oblong to cylindrical and small in size interms of its cell length and breadth.

Description: Epiphyllous, sub-aerial, heterotrichous, prostrate and erect system, rudimentary and pseudoparenchymatous prostrate system. Profuse branching in the main axis, regular branching pattern, oppositely arranged, rarely alternate, lateral branches arranged monopodially, uniseriate axes, 10–14 µm internodal length, apical cells fusiform to blunt, 14–15 µm long, oblong to cylindrical vegetative cell, 10–22 µm long, 3–4 µm wide, thickened cell wall and septum, globular zoosporangium, 3 to 4 µm wide and globular gametangia. The thallus is heterotrichous with the prostrate and erect system. Prostrate part of the thallus is pseudoparenchymatous which creep over the host leaves. Erect filaments profusely branched; each cell is cylindrical to oblong with 10–22 µm long and 3–4 µm wide. Cells of the prostrate system are elliptic or globular; Zoosporangium and gametangium are globular in nature.

Notes: The thallus of alga adherent on the leaves as orange in colour which was usually examined at the host exposed to full sunlight.

Affinities: It closely resembled with *Trentepohlia sundarbanensis* (G.G. Satpati & R. Pal) and *T. arborum* (C. Ag.) Hariot and *T. abietina* (Flotow & Kützing) Hansgirg. One of the unique features of the new species is its profuse branching, branching pattern of main axes (Figs. 3A, D & H) and lateral branches (Figs. 3C, E & G), the shape of apical cells (Figs. 3B, C, F & H), cells length and width (Table 1).

Etymology: The specific epithet ‘*gaviensis*’ was given to signify the place where the specimen was collected. Gavi is one of the pristine and biodiversity-rich natural forests in Pathanamthitta district, Kerala, India.

DISCUSSION

The present study investigated the diversity and taxonomy of *Trentepohlia* spp. on the phyllosphere of rural and semi-urban areas of Kerala, part of the Southwestern Ghats. Eight species of *Trentepohlia* were repeatedly observed on the leaves of host plants in semi-urban areas which were exposed to full sunlight; rare in the natural forest with covered canopy. Interestingly it was found that the new species *Trentepohlia gaviensis* has been examined from the rural area of Kerala covered with natural forest. Species diversity was higher in the semi-urban and low in the rural areas. The present finding suggests that Southwestern Ghats is still an understudied area of epiphyllous *Trentepohlia*. However, the extensive diversity survey was also reported in India (Bruhl and Biswas, 1923; Krishnamurthy, 2000; Saxena, 1961; Randhawa and Venkataraman, 1962; Jose and Chowdary, 1980; Satpati *et al.*, 2013; Satpati and Pal, 2015, 2016). In Kerala, altogether eight species of *Trentepohlia* were examined from rock and bark substratum (Panikkar and Sindhu, 1993). All these investigations are focused on the isolation of *Trentepohlia* either from artificial substrata or from the bark of the trees. In the previous report (Rindi *et al.*, 2006; Rindi and Lopez-Bautista, 2008) *T. abietina* had been examined from different substrates in a different environment including city and forest. *T. chapmanii* was found growing on trees in urban environments. *T. arborum* was found growing on artificial substrata. *T. aurea* showed no such preference at all. Rindi and Guiry (2002) recorded 27 aerophytic green algae in urban environments of Ireland. But the present study exclusively from the phyllosphere of vegetation. All the studied algae have a green colour in the young stage; however, they attain a red colour in the mature stage. Previous studies investigated that the red colour of the *Trentepohlia* is due to the presence of carotenoids (Czeczuga, 1977). Epiphyllous *Trentepohlia* can withstand diverse environmental conditions owing to these unique features. Carotenoids have been assigned a protective function against insolation (Czeczuga, 1977) and resist strong UV radiation in the high altitude mountain region. The genus is also rich in oils which can tide over the cold dry winter. (Gildemeister, 1916). Presence of sporopollenin-like components (Good and Chapman, 1978) and a special carbohydrate and alcohol pattern (Feige and Kremer, 1980; Patterson and Valkenburg, 1991) in the cell wall helps to adapt in the extreme environmental conditions. The present investigation revealed that *Trentepohlia* is adapted to live on the phyllosphere in different environmental conditions. The reported new species was isolated from the dense forest region of Kerala. It was supported by the

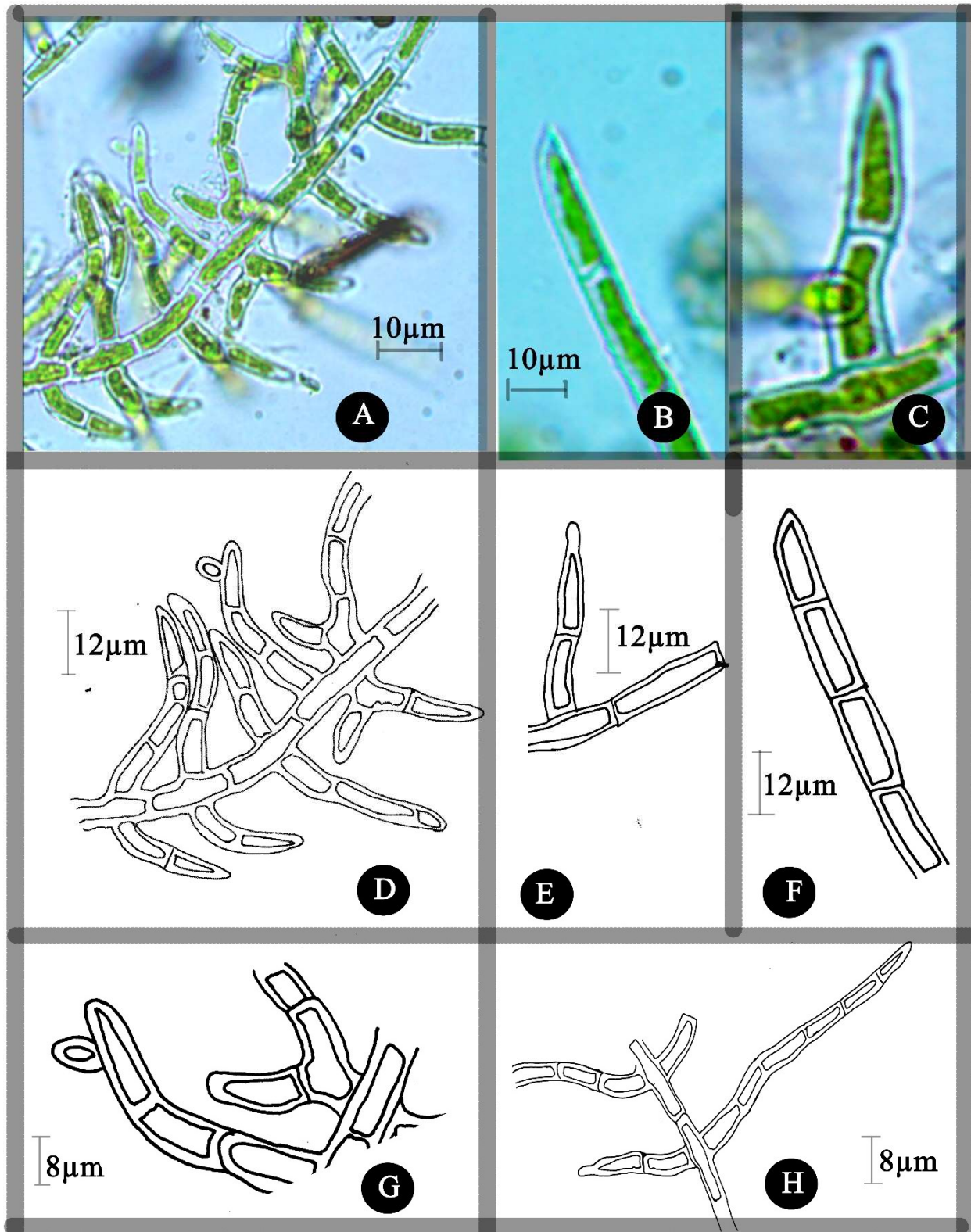


Fig. 3. Microphotographs of *Trentepohlia gaviensis* sp.nov; A-Thallus & branching pattern; B-C. Shape of apical cells; D-H-Line drawings of *T. gaviensis*. sp.nov., D. Thallus with sessile sporangia. E & F. Various shape of the apical cells, G. Side branches with sessile sporangium. H. Opposite branching.

**Table 1:** Comparison of *Trentepohlia gaviensis*, sp. nov. with *T. sundarbanensis*, *T. arborum* and *T. abietina*

| Features | <i>Trentepohlia gaviensis</i> , sp nov | <i>T. sundarbanensis</i> | <i>T. arborum</i> | <i>T. abietina</i> |
|-----------------------|-------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------|--------------------------------------------|----------------------------------------------|
| Habit | Heterotrichous, rudimentary prostrate axes | Heterotrichous, clear distinction between prostrate and upright system | Heterotrichous, limited prostrate axes | Heterotrichous, prostrate system is reduced |
| Habitat | Epiphyllous on <i>Pavetta indica</i> L. | Epiphytic on tree trunk of <i>Avicennia alba</i> | Epiphyllous and corticolous | Epiphyllous and corticolous |
| Branching | Profuse and regular branching pattern in main axis, opposite. Rarely alternate branches seen, Lateral braches in monopodial fashion | Irregular branching pattern | Poorly branched | Little or not branched |
| Apical cell | Fusiform to blunt | Blunt | Blunt to pointed | Usually longer, slightly tapering |
| Cells shape | Oblong to cylindrical | Cylindrical to swollen | Cylindrical | Cylindrical to barrel shaped |
| Nature of cell wall | Thicker | Thicker | Smooth | Smooth |
| Cell length | 10–22 µm | 50–80 µm | 34–78 µm | 18–20 µm |
| Cell breadth | 4–16 µm | 30–40 µm | 10–34 µm | 5–10 µm |
| Sporangia | Sessile, globular, lateral | Stalked, globular, lateral or terminal | Elliptic or spherical, terminal or lateral | Globular, both terminal and lateral, sessile |
| Diameter of sporangia | 3–4 µm | 50–80 µm | 17–19 µm | 14–15 µm |

views of Rindi and Guiry (2002) and Rindi and Lopez Bautista (2008). According to them *Trentepohlia* in its natural habitats compete for light and moisture and allows to produce more biomass in a limited space. *Trentepohlia* alternate between a period of exposure to strong light and immersion by rain in their natural habitats. Therefore, *Trentepohlia* exhibits the ability to survive in aerial conditions. (Chen *et al.*, 2014). Species of *Trentepohlia* is economically important due to the presence of antioxidants and antimicrobial active compounds in their cells (Chen *et al.*, 2014; Simic *et al.*, 2016). According to Abe *et al.* (2002). *T. aurea* has the capacity of removing nitrogen and phosphorous from water. Considering their ecological and economical importance, more exploration of *Trentepohlia* spp. from the Southwestern Ghats is necessary for further research.

CONCLUSION

The present study examined the algae *Trentepohlia* on the phyllosphere of Southwest Ghats. The study revealed that algae are highly diverse and the region is a major habitat of *Trentepohlia*; 13% of the reported species were new to the science and 38 % was a new record of Kerala, India. Considering their potential applications, extensive studies are required for discovering and documenting more valuable species from the study area. Further studies based on 18S rRNA sequencing of *Trentepohlia* will be useful to unravel the interrelationship between the species within the genus.

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