

## Two new records of Sri Lankan hornworts, *Notothylas javanica* (Notothyladaceae) and *Megaceros flagellaris* (Dendrocerotaceae)

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**Abstract** – Sri Lankan hornworts remain the least studied group of bryophytes. So far, three families (Anthocerotaceae, Notothyladaceae, Dendrocerotaceae) and 4 genera (*Anthoceros*, *Folioceros*, *Phaeoceros* and *Dendroceros*) have been recorded within the country. During the present study, hornworts were freshly collected from different geographical localities within the country and identification was done using light and stereo-microscopy and SEM spore morphological observations. Based on this study, *Notothylas javanica* (Sande Lac.) Gottsche (Notothyladaceae) and *Megaceros flagellaris* (Mitt.) Steph. (Dendrocerotaceae) are reported for the first time in Sri Lanka. The species are described and illustrated with notes on their ecology and distribution.

**Sri Lanka / Hornworts / Notothyladaceae / *Notothylas javanica* / Dendrocerotaceae / *Megaceros flagellaris***

### INTRODUCTION

Sri Lanka is a tropical island in the Indian Ocean with a total land area of 65, 610 km<sup>2</sup> (Gunawardene *et al.*, 2007) and harbours a vast diversity of ecosystems. The different climatic zones and diverse topography of the country make Sri Lanka a biodiversity hotspot (Gunawardene *et al.*, 2007). Studies on cryptogamic plants, especially on bryophytes, in Sri Lanka are scarce and confined mainly to the 19<sup>th</sup> century collections by George Gardner (1843-1849), G.H.K. Thwaites (1850-1880), W. Mitten (1819-1906), and some recent accounts (Herzog, 1911; Hattori, 1968; Onraedt, 1986; O’Shea, 2003). Many localities in Sri Lanka, including natural forest and threatened ecosystems (especially in the lowland

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Table 1. Families, genera and species of hornworts in Sri Lanka (based on Long and Rubasinghe, 2014)

Family	Genera	Species
Anthocerotaceae	<i>Anthoceros</i> L.	<i>A. agrestis</i> Paton <i>A. erectus</i> Kashyap
	<i>Folioceros</i> D. C. Bharadwaj	<i>F. amboinensis</i> (Schiffn.) Piippo
	<i>Phaeoceros</i> Prosk.	<i>P. carolinianus</i> (Michx.) Prosk.
Notothyladaceae	<i>Phaeoceros</i> Prosk.	<i>P. carolinianus</i> (Michx.) Prosk.
Dendrocerotaceae	<i>Dendroceros</i> Nees.	<i>D. borbonicus</i> Stephani

wet zone of the country), remain under-explored for bryophytes. Amongst bryophytes, hornworts (Phylum Anthocerotophyta) are the least studied. There are very few herbarium collections in the National Herbarium Peradeniya (PDA), which are in poor condition and usually misidentified. According to the latest checklist of liverworts and hornworts of Sri Lanka by Long and Rubasinghe (2014) only 4 genera and 5 species of hornworts are present in Sri Lanka (Table 1).

During the present study we report the occurrence of *Notothylas* Sull. ex A. Gray (*N. javanica* (Sande Lac.) Gottsche. (Notothyladaceae) and *Megaceros* Campb. (*M. flagellaris* (Mitt.) Steph. (Dendrocerotaceae), two new hornwort genera and species to Sri Lanka.

Out of the ten genera of hornworts recognized worldwide today, *Notothylas* Sullivant (1846:74) (Notothyladaceae) is the fourth largest genus consisting of about 22 species, distributed in tropical and temperate regions (Renzaglia *et al.*, 2009; Villarreal *et al.* 2014; Chantanaorrapint, 2014). William Starling Sullivant (1803-1873) in his “Musci and Hepaticae of the Eastern United States” introduced the genus *Notothylas* for the first time based on *N. orbicularis* (Sullivant, 1846; Underwood, 1882; Chantanaorrapint, 2015). *Notothylas* differs from all other hornworts by its small and solid thallus and reduced, horizontally lying sporophytes (Renzaglia, 1978; Singh 2002). The abbreviated growth of the sporophyte is characteristic of *Notothylas*. The sporophyte lacks stomata, and remains mostly enclosed within the involucre (Renzaglia, 1978). Unlike most hornwort genera which can be defined by their spore colour (e.g. *Phaeoceros* has yellow spores), species of *Notothylas* can have either yellow or dark brown spores. The presence or absence of a suture, columella and pseudoelaters are useful traits to define species within the genus. The combination of those characters is useful to determine species, although much work is needed specially using fresh material (Stieperaere and Matcham, 2007) and modern genetic tools.

The genus *Megaceros* (Dendrocerotaceae) was first described by Campbell in 1907 (Campbell, 1907). *Megaceros* species are widely distributed in tropical and sub-tropical Asia and temperate and tropical rainforests of Eastern Australia (Hasegawa, 1983; Cargill *et al.* 2013). Recent molecular studies have split the genus into two distinct entities; *Megaceros* and *Nothoceros* (R. M. Schust.) J. Haseg. (Villarreal *et al.*, 2010; Cargill *et al.*, 2013). *Megaceros* s.s. is currently known from Africa, Asia and Australasia (Cargill *et al.*, 2013). The main characters of the genus include solid thalli, 1-8 (-12) chloroplasts per cell without pyrenoid, 1 (-2) antheridia per chamber with non-tiered jacket cell arrangement, “green” spores due to large chloroplasts and thin hyaline exine; with mamilllose and/or tuberculate ornamentation and pseudoelaters with helical thickenings. The broadly radiating thalli and greenish and globose spores are the field features of the genus *Megaceros* (Hasegawa, 1983).

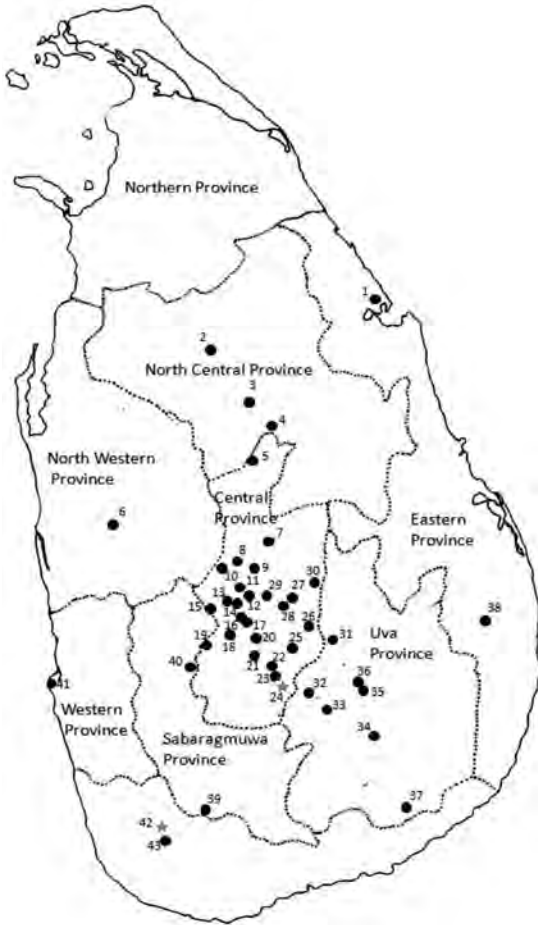


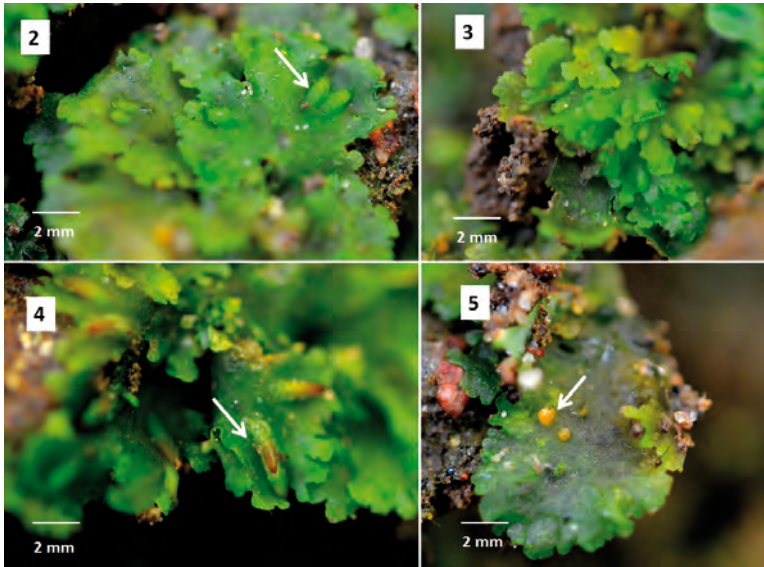
Fig. 1. Map of surveyed localities in Sri Lanka. 1. Trincomalee, 2. Mihintale, 3. Ritigala, 4. Habarana, 5. Dambulla, 6. Paduwasnuwara, 7. Riverstone, 8. Matale, 9. Elkaduwa, 10. Karagahapitiya, 11. Watapuluwa, 12. Thennekumbura, 13. Peradeniya, 14. Hanthana, 15. Kadugannawa, 16. Nilambe, 17. Galaha, 18. Atabage, 19. Dolosbage, 20. Loolecondera, 21. Ramboda, 22. Pidurutalagala, 23. Nuwara Eliya, 24. Hapkgala, 25. Walapane, 26. Randenigala, 27. Hunnagiriya, 28. Medamahanuwara, 29. Teldeniya, 30. Hasalaka, 31. Badulla, 32. Welimada, 33. Bandarawela, 34. Pelwatta, 35. Passara, 36. Pelgahatenna, 37. Kataragama, 38. Ampara, 39. Sinharaja, 40. Kitulgala, 41. Colombo, 42. Hiniduma, 43. Kanneliya.

## MATERIALS AND METHODS

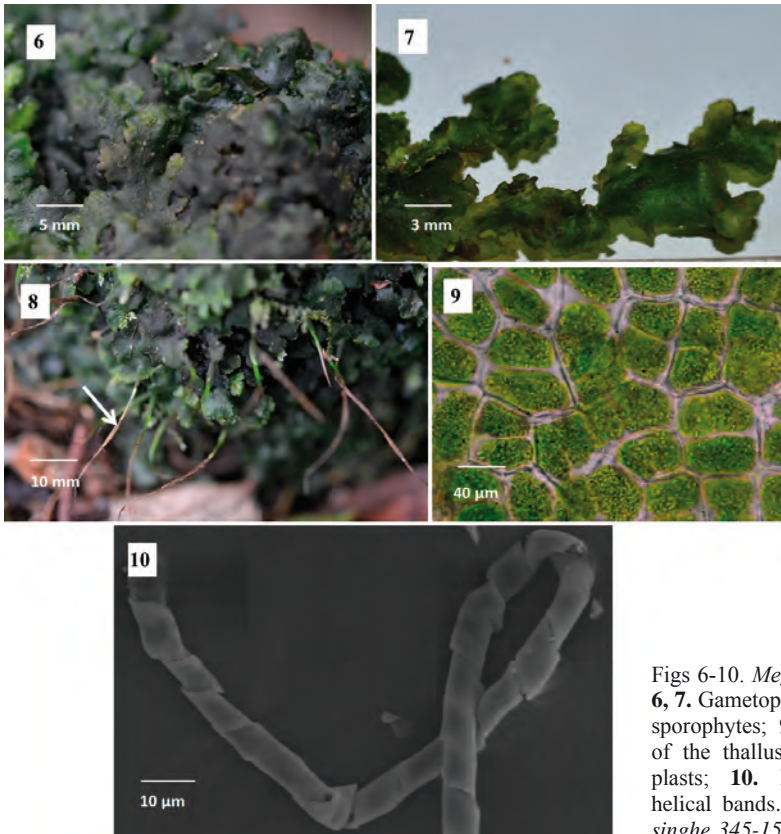
Fieldwork was carried out in different geographical localities throughout the country (Fig. 1). Samples of the fresh thalli and sporophytes were studied using a compound light microscope and stereo-microscope (Olympus CX21, Eruomex, Arnhem, Holland) to illustrate the distinctive characters of the species. Spores were studied using scanning Electron Microscopy (SEM) (Evo LS 15 Zeiss, Germany).

## RESULTS AND DISCUSSION

We provide descriptions of the new records to Sri Lanka, all measurements are based on our material and their identity verified with type specimens.



Figs 2-5. *Notothylas javanica*. 2, 3. Gametophyte (thallus) with immature horizontally lying sporophytes; 4. Mature sporophytes partially covered by the involucre; 5. Thallus with yellow antheridia. Ruklani & Rubasinghe, 308-15.

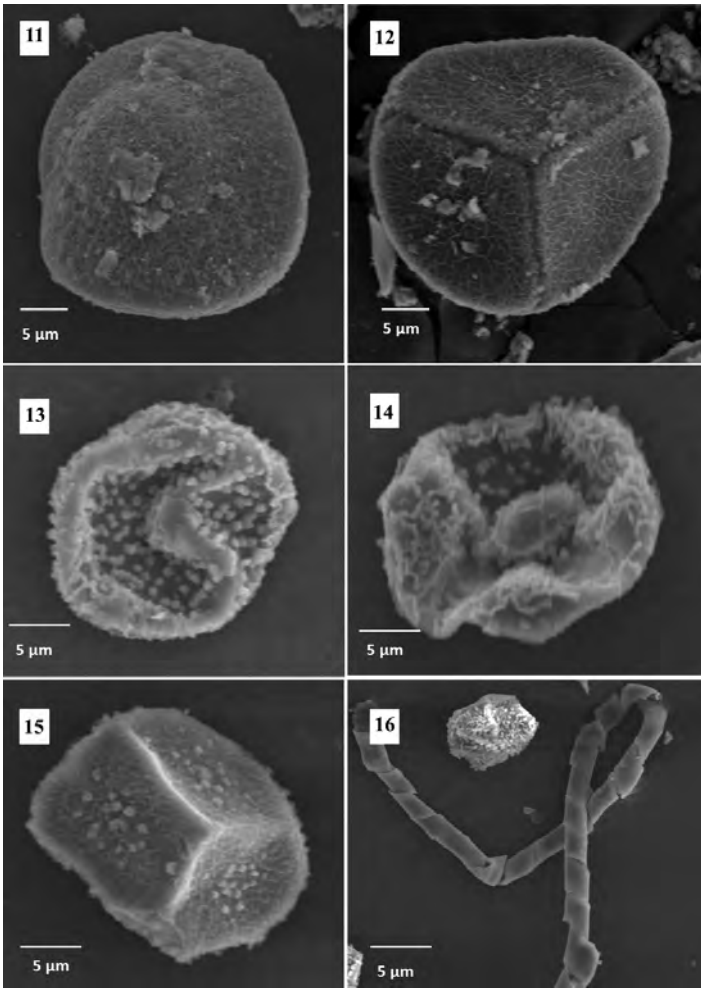


Figs 6-10. *Megaceros flagellaris*. 6, 7. Gametophyte; 8. Thalli with sporophytes; 9. Epidermal cells of the thallus with 1-4 chloroplasts; 10. Pseudoelater with helical bands. Ruklani & Rubasinghe 345-15.

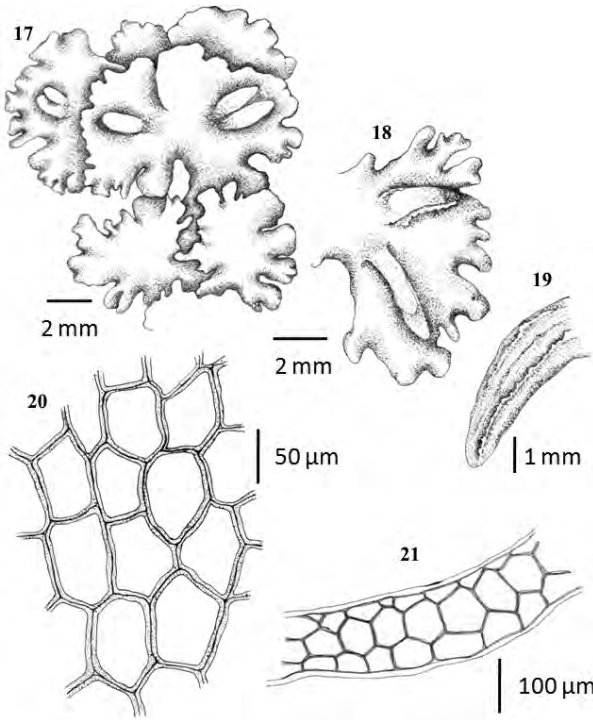
*Notothylas javanica* (Sande Lac.) Gottsche. *Botanische Zeitung (Berlin) Beil.* 16: 20. 1858 **Figs 2-5, 11-12, 17-20**

≡ *Blasia javanica* Sande Lac. *Synopsis Hepaticarum Javanicarum*: 94, 1856 [1857] (Sande Lacoste 1856). Type: [Java] Van der Sande Lacoste 1857. (Holle in Hb. Dozy et Mb.). Tabula XIX f. 1-4, 11.

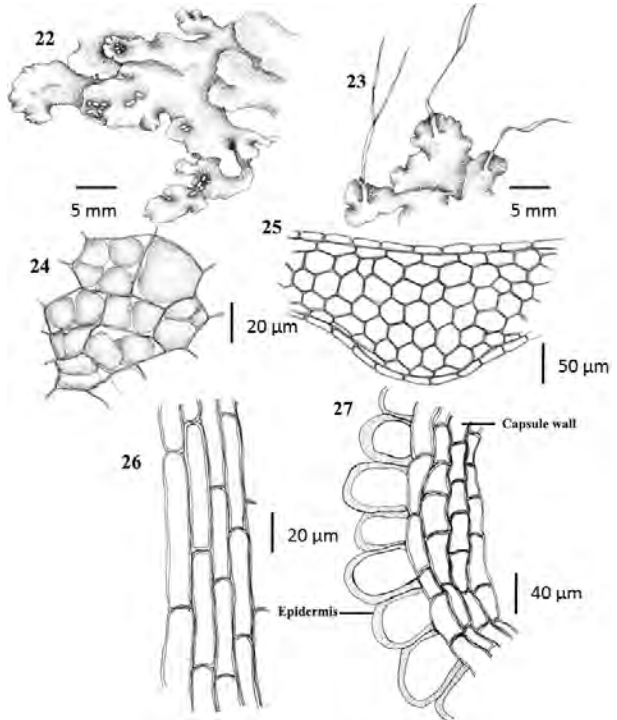
**Description:** Thalli 2.0-8.0 mm in total width, forming rosettes, yellowish green to green, margins with imbricate lobes, epidermal cells irregularly hexagonal. Involucre asymmetrical, rough, lamellate slightly lacinate towards the tip. Monoicous. Androecia scattered, antheridia 2-4 per chamber with non-tiered jacket cell



Figs 11-16. *Notothylas javanica*. **11.** Distal face of the spore showing finely vermiculate ornamentation; **12.** Proximal face of the spore. *Ruklani & Rubasinghe, 308-15. Megaceros flagellaris*; **13, 14.** Distal face of the spores showing mamilliose ornamentation; **15.** Proximal face of the spore; **16.** Pseudodelater. *Ruklani & Rubasinghe 345-15.*



Figs 17-21. *Notothylas javanica*.  
**17, 18.** Thalli with sporophytes;  
**19.** Mature sporophyte with lamellate involucre;  
**20.** Cells of the sporophyte with moderately thickened cell walls;  
**21.** Transverse section of the thallus. *Ruklani & Rubasinghe, 308-15.*



Figs 22-27. *Megaceros flagellaris*.  
**22.** Gametophyte; **23.** Gametophyte with sporophytes;  
**24.** Epidermal cells containing one to multiple large chloroplasts;  
**25.** Transverse section of the thallus;  
**26.** Epidermal cells of the sporophyte;  
**27.** Transverse section of the sporophyte. *Ruklani & Rubasinghe 345-15.*

arrangement. Capsules small, 1.0-2.0 mm long, oblong cylindrical, irregularly ruptured not valved, epidermal cells long rectangular with moderately thick walls, columella absent. Spores yellow, spherical to tetrahedral, 40-45  $\mu\text{m}$  in diameter, proximal surface finely vermiculate, distal surface with a slightly dome-like region at the centre. Pseudoelaters absent.

**Specimen examined:** Sri Lanka. Southern Province, Galle District, Hiniduma (6° 19' 2.1714" N, 80° 19' 0.9114"E), Alt. 9m. May 27, 2015. On wet soil, very humid area, home garden. *Ruklani & Rubasinghe, 308-15* (PDA).

*Notothylas javanica* differs from other *Notothylas* species by the irregularly ruptured capsule wall without any special row of thick walled cells, moderately thick-walled and irregularly arranged epidermal cells of the capsules, absence of pseudoelaters and distinctly lamellate and laciniate involucre (Hasegawa, 1979). According to Chantanaorrapint (2015) this species shares its spore sculpturing and the shape of the epidermal capsule wall cells with the African *N. decurva* (Mitt.) Steph. However, *N. javanica* differs from *N. decurva* in the absence of pseudoelaters (Stieperaere & Matcham, 2007; Chantanaorrapint, 2015). *N. javanica* shares similar characteristics with *N. orbicularis* (Schwein.) Sull. ex A. Gray in sporoderm architecture (Chantanaorrapint, 2015). *Notothylas javanica* differs from *Notothylas orbicularis* by lacking dehiscent bivalved capsules with strongly thickened epidermal cells, and the absence of pseudoelaters (Hasegawa, 1979; Stieperaere & Matcham, 2007; Chantanaorrapint, 2015). Chantanaorrapint (2014) records *Notothylas irregularis* as a new species under the genus from Northern Thailand. This species also lacks pseudoelaters and has a vermiculate spore surface as in *N. javanica*. *Notothylas irregularis* Chantanaorr. differs from *N. javanica* by having a central depression on each proximal surface (Chantanaorrapint, 2014). The vermiculate surface of the spores is also shared by the Indian *Notothylas* species, *N. indica* Kashyap and *N. himalayensis* Udar & D. K. Singh (Udar & Singh, 1981; Singh, 2002; Chantanaorrapint, 2015). The distinctions among these species are summarized in the Table 2.

**Distribution:** Southern Japan, Formosa, Philippines (Luzon) and Indonesia (Java) (Hasegawa, 1979), China, Congo, Thailand, Panama (Dauphin *et al.*, 2006; Chantanaorrapint, 2015; Stieperaere and Matcham, 2007). The species is typically associated with man-made habitats and typically found in gardens and near houses.

*Megaceros flagellaris* (Mitt.) Steph., *Spec.Hep.* 5:951, 1916 **Figs 6-10, 13-14, 22, 27**

≡ *Anthoceros flagellaris* Mitt. in Seemann, *Flora Vitiensis* 419. 1873. Type specimen: [Samoa], *R. T. Powell s.n.* (Lectotype designated by Proskauer, NY!).

**Description:** Thalli solid, flat, dark green to brownish green when moist, brownish when dry, up to 5 cm long, more or less dichotomously branched, branches 5-8 mm in width. Margins of the branches undulate. Epidermal cells quadrate to hexagonal with 1-4 chloroplasts without pyrenoids. Monoicous. Androecia in lateral branches and main thallus. Antheridia 1-2 per chamber, single, globose, short pedicellate. Involucre solitary, 5-8 mm in length, erect, cylindrical. Capsules 4-6 cm long. Epidermal cells rectangular to elongate-linear. Spores greenish due to the large chloroplast and hyaline spore wall, globose to rounded tetrahedral, 15-20  $\mu\text{m}$  in diameter, densely papillose on proximal surface. Pseudoelaters pale brown, with helical bands.

**Specimen examined:** Sri Lanka. Central Province, Nuwara Eliya District, Hakgala (6° 55' 48.9354" N, 80° 48' 43.416"E), Alt. 1792 m. May 27, 2015. On wet rocks and tree logs, shady area inside forest. *Ruklani & Rubasinghe 345-15* (PDA).

Table 2. Comparison of *Notothylias* species based on capsule, pseudoelaters and spore characters

	<i>N. javanica</i>	<i>N. orbicularis</i>	<i>N. indica</i>	<i>N. himalayensis</i>	<i>N. irregularis</i>
<b>Involucre</b>	Longitudinally lamellate	Distally lamellate	Distally lamellate	Distally lamellate	Longitudinally lamellate
<b>Dehiscense line</b>	Absent	Present	Present	Present	Present
<b>Epidermal cells</b>	Moderately thick walled	Strongly thick-walled	Strongly thick-walled	Sinuate or nodulose thickenings on their radial walls	Moderately thick walled
<b>Epidermal cell shape</b>	Rectangular	Rectangular	Quadrato-subquadrato	Quadrato to sub quadrato towards, sub-quadrato to elongate rectangular	Subquadrato to sub rectangular to rectangular
<b>Columella</b>	Absent	Present	Present	Present	Present
<b>Pseudoelaters</b>	Absent	Present	Present	Present	Absent
<b>Spore size</b>	40-45 µm	33-41 µm	46-66 µm,	30-37 µm	30-35 µm
<b>Spore colour</b>	Yellowish	Yellowish to pale brown	Dark brown	Yellowish-deep brown	Yellowish to pale brown
<b>Spore Ornamentation</b>	Proximal face vermiculate without a central hollow. Distal face convex with a central hump-like structures	Proximal facevermiculate without a central hollow. Distal face convex with a central hump-like structures	Proximal facevermiculate without a central hollow. Distal face convex with a central hump-like structures	Proximal face vermiculate without a central hollow but a poorly developed, wavy tri-radiate mark. Distal face convex with a central hump-like structure	Proximal face vermiculate with a central hollow. Distal face convex without central hump-like structures

The only revision of the Asiatic *Megaceros* species synonymized 16 names under *M. flagellaris* (Hasegawa, 1983). He concluded that a single very plastic species occurs throughout Asia and potentially Oceania. The diagnostic features of *Megaceros flagellaris* are spore characters; the central bump in the distal face and the tessellate pattern in the equatorial region (Villarreal *et al.*, 2010; Cargill *et al.*, 2013) and the uniformly distributed verrucae on each triangular proximal face (Cargill *et al.*, 2013). Cargill *et al.* (2013) have assessed the monophyly of the Australian and New Zealand *Megaceros* that share the same spore and gametophyte morphology. Species with similar spore sculpture in Australia and New Zealand (*M. austronesophilus* Cargill & Seppelt and *M. gracilis* (Reichardt) Steph.) were distinguished by their sexual condition, *M. austronesophilus* is dioicous, while *M. gracilis* is monoicous. The main distinction between *M. gracilis* and *M. flagellaris* lies on the spore ornamentation. Spores of *M. flagellaris* present a tessellate patterning around equatorial girdle whereas spores of *M. gracilis* consist of tubercles or ribbing around circumference of distal face (Fig. 2, Cargill *et al.*, 2013). The spore



morphology of the species of *Megaceros* encountered during the present study differs slightly from that of *M. flagellaris* by lacking the distinct tessellate patterning around equatorial girdle. However almost all the gametophytic characters of the specimen studied are similar to those of *M. flagellaris*. We are currently conducting molecular phylogenetic analysis of the collection to further confirm the identity of the species.

**Distribution:** China, Japan, India, Thailand, Philippines, Borneo, Indonesia, Papua New Guinea, New Caledonia, Samoa, Society Islands, Hawaii, Vanuatu, Australia and New Zealand and Tanzania (Campbell, 1984; Hasegawa, 1986; Hasegawa, 2002; Vella & Cargill, 2003; Garcia *et al.*, 2012).

### CONCLUDING REMARKS

*Notothylas* and *Megaceros* are two new generic records to Sri Lanka. This work highlights the need for more collections of hornworts within the country and to provide more localities and information on the natural history of these species for further biogeographical work.

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