

LEANDRO DE ALMEIDA AMÉLIO

Circunscrição e filogenia de *Notothylas* Sull. (Notothyladaceae, Anthocerotophyta)

Dissertação apresentada ao Instituto de Botânica da Secretaria do Meio Ambiente, como parte dos requisitos exigidos para a obtenção do título de MESTRE em BIODIVERSIDADE VEGETAL E MEIO AMBIENTE, na Área de Concentração de Plantas Avasculares em Análises Ambientais.

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ORIENTADOR: DR. DENILSON FERNANDES PERALTA

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*Dedico a todos os briólogos, alunos e profissionais
que se dedicam a estudar esses lindos organismos.*

“Many a botany student has had it explained to him that the bryophytic way of life is rather a poor idea without a glorious future in the full exploitation of the land habitat.... On the contrary, it is an excellent idea with so much future that the plants which adopted it have rigorously stayed with it, finding ample opportunity for themselves in a succession of geological epochs.”

Proskauer 1964

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Resumo

Os antóceros formam a menor divisão do *lato sensu* de briófitas, é normalmente conhecido por serem plantas talosas com cloroplastos grandes e pirenóide, e geração esporofítica prolongada com estômatos. Análises indicam que o grupo apresenta proximidade filogenética com plantas vasculares. Neste estudo é apresentado o gênero *Notothylas* Sull. para o Brasil (Capítulo 1) e para o mundo (Capítulo 2), com sinônimos novos, descrição de duas espécies novas e comentários filogenéticos. Além disso, também é apresentado a biogeografia do gênero para os dois contextos e a resolução de conflitos taxonômicos que pairam sobre o gênero desde 1992, com a divisão em dois subgêneros por Asthana & Srivastava e Schuster, utilizando ferramenta molecular e análise cladística. Foram analisadas cerca de 100 amostras incluindo tipos nomenclaturais depositados em herbários nacionais e internacionais. Como primeiro resultado apresentamos a revisão das espécies de *Notothylas* para o Brasil, diferenciando-as através das características morfológicas do esporófito (cápsula, esporo, columela, pseudo elatério, invólucro) e do gametófito (talo, rizóides, cloroplasto, associação com *Nostoc* cianobactérias). Como principais resultados estão a proposição da sinonimização de *Notothylas vitalii* (Udar & Singh) e a descrição de duas novas espécies para a ciência. Como segundo resultado, é apresentada uma sinopse mundial do gênero *Notothylas*, elaborada através de pesquisa histórica das classificações mais antigas e contemporâneas e de análise morfológica. Visamos apresentar um tratamento taxonômico incluindo chaves de identificação, descrições, ilustrações, contemplando a biogeográfica, juntamente com comentários filogenéticos sobre os subgêneros. Como principais resultados estão a sinonimização do subgênero *Notothyladoides* a exclusão de espécies duvidosas para o gênero como *Notothylas minuta*, os comentários filogenéticos gerados a partir de ferramentas moleculares, as ilustrações e o tratamento taxonômico.

Palavras chave: Antóceros, chave de identificação, revisão taxonômica, espécie nova, comentários filogenéticos.

Abstract

The hornworts are the smaller division of *lato sensu* of bryophytes, normally is known by thallus plants with big chloroplasts and pyrenoid, the sporophyte generation is prolong with presence of stomata. The group present approximate phylogeny with vascular plants. In this study, we present the genus *Notothylas* for Brazil (Chapter 1) and to the world (Chapter 2), with news combinations, description of two news species and phylogenetic comments. Besides that, the biogeography to genus and the resolution of taxonomic conflicts about the genus since 1992, with the division in two subgenus by Asthana & Srivastava, and Schuster, using molecular tools and cladistics analyses. Around 100 samples were checked including nomenclature types deposited in national and international herbariums. As the first result we present a review of the species of the *Notothylas* to Brazil, differ by the morphology characters of the sporophyte (capsule, spore, columella, pseudoelater, involucre) and of the gametophyte (thallus, rhizomes, chloroplast, and *Nostoc* disposition). As main results are the proposition of the synonymy of *Notothylas vitalii* (Udar & Singh) and the description of two news species to science. To second result, we present a world synopsis of the genus *Notothylas* elaborated through historical research of the oldest and contemporary classifications and morphological analysis. We aim to present a taxonomic treatment including identification keys, descriptions, illustrations, contemplating the biogeographic, along with phylogenetic comments on the subgenera. As main result are the sinomization of the subgenus *Notothyladoides* the exclusion of dubious species for the genus as *Notothylas minuta*, phylogenetic comments generated from molecular tools, illustrations and taxonomic treatment.

Key words: Hornworts, identification key, taxonomic review, new specie, phylogenetic comments.

LISTA DE FIGURAS

- Figura 1. A-C. Aspectos dos talos. A. Talo flabelado desidratado em exsicata de *Notothylas flabellata*. B. Talo flabelado desidratado em exsicata de *Notothylas breutelii*. C. Talo rosulado em habitat natural de *Notothylas orbicularis*. D. Anterídio: Invólucro: 5-involucro com alguns esporos provenientes da cápsula. E-F. Aspectos das cápsulas. E. Cápsula valvada, com linha de deiscência. F. Cápsula, não valvada, sem linha de deiscência 69
- Figura 2. A-C. Aspectos das columelas. A. *Notothylas breutelii*. B. *Notothylas temperata*. C. *Notothylas orbicularis*. D-G. Aspectos dos esporos. D. Baculado. E. Vermiculado. F. Tuberculado. G. Esporos ainda em tétrade. H-I. Aspectos dos pseudoeláterios. H. Pouco desenvolvido. I. Maduro. 70
- Figura 3. A. Distribution range of *Notothylas* in Brazil. B-H. *Notothylas breutelii* (Gottsche) Gottsche. B. Thallu with sporophytes. C. Capsule margin. D. Collumela. E. Spore dorsal view. F. Spore proximal view. G. Pseudoelater. H. Type of *Notothylas cubana* 71
- Figura 4. A-E. *Notothylas javanica* (Sande Lac.) Gottsche. A. Thallu with sporophyte. B. Capsule with spores. C. Spore proximal view. D. Spore dorsal view. E. Type specimen. F-L. *Notothylas orbicularis* (Schwein.) Sull. F. Thallu with sporophyte. G. Capsule with spores. H. Collumela. I. Spore dorsal view. J. Spore proximal view. K. Pseudoelater. L. Type specimen 72
- Figura 5. *Notothylas granulata* Amélio & Peralta. A. Rehydrated plant. B. Distal cells of the capsule. C. Light microscopy of the proximal spore surface. D. Light microscopy of the distal spore surface. E-G. Pseudoelaters. H. SEM of the proximal spore surface. I. Detail of the ornamentation of the hollow on the proximal face. J. SEM view of the distal face of the spore. K. Detail of the ornamentation on the distal surfasse 73
- Figura 6. *Notothylas vermiculata* Amélio & Peralta. A. Rehydrated plant. B. Distal cells of the capsule. C. Light microscopy of the proximal spore surface. D. Light microscopy of the distal spore surface. E-F. Pseudoelaters. G. SEM of the proximal spore surface. H. Detail of the ornamentation of the hollow on the proximal face. I. SEM view of the distal ace of the spore. J. Detail of the ornamentation on the distal surfasse 68
- Figura 7. Cluster analysis of all 21 recognized species concerning about the morphological structures, abbreviation of species and data on based on ANEX III. 69
- Figura 8. A. Cladogram based on a maximum parsimony analysis of *Notothylas* based on rbcL. B. Cladogram based on a maximum likelihood analysis of *Notothylas* based on rbcL. 70
- Figura 9. Filogram based on a maximum Bayesian analysis of *Notothylas* based on rbcL. 71
- Figura 10. A-F. *Notothylas dissecta* Steph. A. Thallu with sporofites. B. Capsule margin. C. Collumela. D. Spore proximal view. E. Spore dorsal view. F. Type of *Notothylas dissecta*..... 72
- Figura 11. A-F. *Notothylas flabellata* Steph. A. Thallu with sporophytes. B. Capsule with spores. C. Spore proximal view. D. Spore dorsal view. E. Pseudoelater. F. Type of *Notothylas flabellata*..... 73
- Figura 12. A-G. *Notothylas temperata* J. Haseg. A. Thallu with sporophytes. B. Capsule. C. Collumela, D. Spore dorsal view. E. Spore proximal view. F. Pseudoelater. G. Type of the *Notothylas temperata* 74

Sumário

1. Introdução Geral	13
1.1. Visão Geral	13
1.2. História taxonômica do gênero <i>Notothylas</i>	15
a. Talo	16
b. O Cloroplasto	17
c. Invólucro	18
d. Esporângio	18
e. Cápsula	18
f. Columela	19
g. Esporos	19
h. Pseudo elatérios	20
2. Objetivos	20
3. Materiais e métodos	20
4. The genus <i>Notothylas</i> (Notothyladaceae, Anthocerotophyta) in Brazil	22
Introduction	22
Materials and methods	24
Results and discussion	24
Taxonomic treatment	25
Key to species of <i>Notothylas</i> in Brazil	25
4.2. A world synopsis of the genus <i>Notothylas</i> Sull.	34
(Notothyladaceae, Anthocerotophyta) with phylogenetic comments	34
Introduction	35
Materials and Methods	36
Morphological delimitation	36
Molecular study	37
Results and Discussion	39
Taxonomic treatment	42
Considerations	58
References	60

5. Illustrations	68
6. ANEXOS	80

LISTA DOS ANEXOS

Anexo I. Cronologia do surgimento e descrição das espécies dentro do gênero <i>Notothylas</i> ..	81
Anexo II. Table 1. Morphological comparison of Brazilian <i>Notothylas</i> species	84
Anexo III. . Primers used in this study, daggers (†) are for primers using only for sequencing	86
Anexo IV. Morphological data used in the cluster analysis. A. Gametophyte form, B. Presence of pyrenoid, C. Spore color, D. spore ornamentation, E. Conical projection on outer surface, F. Presence of pseudo elater, G. Presence of columela, H. Presence of valva in capsulae, I. Number of cell layer in the valava, J. Depression inner spore, K. Spore wide group, L. Spore long group.....	86
Anexo V. Description of the sequences of mark rbcL, of the analysis CI: consistency index and CR: retention index	87

1 Introdução Geral

1.1 Visão Geral

As briófitas formam um grupo altamente diversificado de plantas, com ampla distribuição e conseguindo colonizar todos os ecossistemas terrestres. As briófitas *lato sensu* constituem o segundo maior grupo de plantas terrestres (Buck & Goffinet, 2000), menor em número apenas que as Angiospermas. Morfologicamente estão agrupadas em três divisões: Anthocerotophyta (Antóceros), Marchantiophyta (Hepáticas) e Bryophyta (Musgos) (Buck & Goffinet, 2000), e filogeneticamente são linhagens parafiléticas.

A relação filogenética amplamente aceita entre as três divisões é de que hepáticas, musgos e antóceros são polifiléticos (parafiléticos entre si) e que os antóceros formam um grupo irmão das plantas vasculares. Anthocerotophyta é considerada filogeneticamente o grupo com maior proximidade com as plantas vasculares tanto molecular (Villarreal *et al.*, 2015) quanto em sequências de proteínas (Szövényi *et al.*, 2015). Este grupo é morfologicamente bem estabelecido e geralmente, percebido como muito mais homogêneo e bem delimitado do que musgos e hepáticas, apresenta centros de diversidade em locais remotos, e ocorrência rara na natureza.

Os antóceros são caracterizados pelo talo sem diferenciação de tecido, e pelos grandes cloroplastos com a presença de pirenóides. Essa morfologia também define o grupo em uma posição isolada nas embriófitas (Vaughn *et al.*, 1992), formando um grupo irmão das plantas vasculares. São plantas talosas, com relação simbiótica com cianobactérias *Nostoc*, rizóides unicelulares, células com canais, tilacóides e pirenóides, cápsulas cilíndricas, com ou sem estômatos, columela presente ou ausente, pseudo elatérios presentes ou ausentes, esporos verdes, amarelos, marrons e pretos, com a marca (cicatriz) trilete muito evidente, estão presentes no meio ambiente como saxícolas e terrestres (Stech *et al.*, 2009).

Os antóceros são um grupo extremamente pequeno em número de espécies, a divisão possui atualmente cinco ordens (Anthocerotales Limpricht, Dendrocerotales Hässel, Phymatocerotales R.J. Duff *et al.*, Notothyladales Hyvönen & Piippo e Leiosporocerotales Hässel) que juntas compreendem 12, com cerca de 225 espécies (Söderström *et al.*, 2016).

A taxonomia da divisão Anthocerotophyta está em mudança e atualmente é difícil estimar o número exato de gêneros e espécies, tendo em vista que dos mais de 225 nomes de espécies de antóceros já descritos em todo o mundo, características básicas para o seu posicionamento como descrições detalhadas dos esporos da maioria destes táxons estão

faltando. A ausência de informações é ainda mais alarmante quando pesquisamos espécies que possuem sequências de marcadores disponíveis no GenBank. Estudos para esclarecer as relações taxonômicas entre antóceros baseadas unicamente na morfologia levaram a uma série de conceitos altamente incongruentes de suas inter-relações (Cargill *et al.*, 2005).

Notothyladales é a ordem mais numerosa da divisão Anthocerotophyta, incluindo 63 espécies (mais de um quarto das espécies de antóceros do mundo), nela estão incluídos os gêneros *Notothylas* Sull., *Mesoceros* Piippo, *Paraphymatoceros* Hässel, e *Phaeoceros* Prosk. (Söderström *et al.*, 2016).

O gênero *Notothylas* Sull., foco deste trabalho, apresenta a distribuição geográfica Pantropical, sendo as áreas com maior número de espécies o Sul e o Sudeste asiático, e as áreas próximas ao Equador que apresentam longo período seco, notadamente planícies de sedimentação do quaternário.

Notothylas é caracterizada por plantas talosas, cuja geração esporofítica é extremamente reduzida e por vezes imersa no talo, os esporos apresentam superfície ornamentada ou lisa, com coloração verde, amarela ou marrom, com a presença ou não de pseudo elatérios (Gradstein *et al.*, 2001). O gênero possui como espécie tipo *N. orbicularis* (Schwein.) Sull. e a característica utilizada para a descrição original do gênero foi a cápsula séssil e imersa (Sullivant, 1846).

Atualmente 35 epítetos específicos são conhecidos mundialmente (TROPICOS, 2016). Destes, 21 foram descritos antes de 1950 e são conhecidos somente a partir do material tipo, três são nomes inválidos e somente cinco espécies apresentam completa caracterização morfológica e molecular incluída dentro de uma filogenia, três delas descritas nos últimos dez anos (*Notothylas frahmii* Chantanaorrapint, *N. javanica* (Sande Lac.) Gottsche, *N. orbicularis* (Schwein.) Sull., *N. udarii* D.K. Singh & Semwal e *N. yunnanensis* T. Peng & R.L. Zhu).

Os recentes esforços para aumentar as coleções, e de utilizar técnicas moleculares estão permitindo visualizar um consenso em relação à inter-relações e classificação dos antóceros (Cargill *et al.*, 2005, Duff *et al.*, 2004 e Shaw & Renzaglia, 2004).

Stech *et al.* (2003) e Duff *et al.* (2004) foram os primeiros a apresentar informações obtidas através de técnicas moleculares em antóceros sob a forma de comparações das regiões *trnL-trnF* e *rbcL* do cloroplasto.

A mais abrangente filogenia utilizando *rbcL* proposta por Villarreal & Renzaglia (2015) revelou uma divergência inesperada entre os gêneros *Anthoceros* e *Phaeoceros*, o polifiletismo de *Megaceros*, a ampla divergência de *Leiosporoceros* em relação a todos os outros gêneros, e a existência de um gênero novo com espécies até então incluídas em

Phaeoceros e *Megaceros* (*Phaeomegaceros*).

O genoma dos antóceros é o menor entre todos os grupos de briófitas *lato sensu* e possui cerca de 85 milhões de pares de bases localizados em 5 cromossomos (Szövényi, 2016). As primeiras análises publicadas revelaram a presença de muitos genes únicos, o que é ideal para a análise intra-genérica e específica, prometendo descobertas futuras. Atualmente está sendo investido esforço no cultivo *in vitro* de amostras visando o sequenciado completo do genoma.

Neste trabalho iremos considerar o gênero *Notothylas* incluído na subfamília Notothylatideae da família Notothyladaceae, que constitui um clado monofilético como proposto por Renzaglia *et al.* (2009), com base em estudos morfo-moleculares.

Para o Brasil são conhecidas as espécies *Notothylas breutelii* (Gottsche) Gottsche, *N. orbicularis* (Schwein.) Sull. e *N. vitalii* Udar & Singh., sendo que as duas primeiras apresentam ampla distribuição mundial e *N. vitalii* é endêmica. A distribuição brasileira é na zona tropical, com pouca ocorrência em regiões temperadas, ocorrendo principalmente em áreas abertas.

Este estudo contempla todas as espécies conhecidas de *Notothylas*, uma vez que encontramos incongruências na delimitação entre espécies, além das elencadas como *incert sedis* por Söderström *et al.* (2016) e o baixo número de sequências de DNA que dificulta a discussão das relações filogenéticas intra genéricas no mundo.

1.2 História taxonômica do gênero *Notothylas*

Sullivant (1845) descreveu o gênero *Notothylas* para acomodar *Carpobolus orbicularis* Schwein. (Schweinitz, 1822), um nome novo de *Targionia orbicularis* Schwein. (Schweinitz, 1821), descrito a partir de plantas da Carolina do Norte (U.S.A.).

Dentre as características destacadas na descrição original, apenas “*Capsula involucri inclusa*” indicava uma diferença morfológica consistente para o reconhecimento do gênero. Foi apenas em 1858 que Gottsche, estudando as espécies conhecidas naquela época para o grupo dos antóceros apresentou a primeira circunscrição adequada para o gênero, com descrições e comentários.

Gottsche (1858) reconheceu três espécies deste gênero: *Notothylas orbicularis* (Schwein.) Sull. (tendo *N. valvata* Sull. e *N. melanospora* Sull. como sinônimos); transferiu *Anthoceros breutelii* Gottsche, um táxon descrito para as Antilhas menores do Norte da América do Sul, para *Notothylas breutelii* (Gottsche) Gottsche; e *Blasia javanica* Sande Lac., conhecida de Java, para *N. javanica* (Sande Lac.) Gottsche. Neste mesmo trabalho Gottsche

(1858) organizou estas três espécies em dois grupos com base na morfologia dos esporos, característica que na época foi reconhecida como de grande importância taxonômica para os antóceros

O grupo “*Eu-Notothylas*” agregou as espécies *semina laevia* (em latim '*semina*' = semente e '*laevia*' = lisa) e esporos amarelos, incluindo *N. orbicularis* e *N. javanica* e o grupo “*Acanthonotothylas*” caracterizado como *seminibus muriculatis* (em latim '*seminibus*' = portador de semente e '*muriculatis*' = ornamentos) e esporos pretos, incluindo *N. breutelii* Gottsche. No entanto essa classificação não perdurou, visto que outras espécies foram descobertas e com elas, características como esporo escuro com superfície lisa ou esporo claro com superfície ornamentada foram descritas.

Asthana & Srivastava (1991) ao estudarem as espécies indianas, ampliaram a caracterização morfológica do gênero e propuseram dois novos subgêneros com base na presença ou ausência da columela e pela cápsula ser ou não valvada: subgênero *Notothylas* e subgênero *Notothyloides* Asthana & Srivastava.

Schuster (1992), ao revisar as espécies de hepáticas e antóceros da América do Norte, sinonimizou *N. amazonica* da América do Sul para *Notothylas orbicularis* e *N. cubana* para *N. breutelii*, e também estabelece duas seções infra genéricas; *Notothylas* e *Depressisporae*; e quatro subseções infragenéricas: *Notothylas*, *Acanthonotothylas*, *Flabelatae*, *Anomalae*; utilizando a coloração e ornamentação do esporo para essas classificações.

Ao todo foram publicados 39 nomes de espécies na literatura, as datas e as principais informações de cada publicação estão elencadas no ANEXO I.

1.3. Principais características de importância taxonômica no gênero *Notothylas*

a. Talo

Notothylas apresentam o talo ecostado (sem uma linha mediana delimitada), dorsiventral, geralmente prostrados, algumas vezes dicotômicos, claramente apresentando crescimento em rosetas (Singh, 2002).

O talo típico de *Notothylas* tem colônias de *Nostoc*, um membro de Cyanobacteria, incorporado nos tecidos que fornece uma conversão do nitrogênio atmosférico em uma forma útil ao antóceros. Este nitrogênio é transferido do gametófito para o esporófito. Além disso, se o gametófito passa a ser cultivado no escuro, e o esporófito estiver sendo iluminado, ele pode

transferir a reação fotossintética ao gametófito (Bold *et al.*, 1987). E esse esporófito pode ter o dobro da reação de fixação de carbono do gametófito (Thomas *et al.*, 1978).

O talo apresenta considerável variação na morfologia superficial externa, sendo observada os seguintes padrões: 1. Talos radiados e pequenos, margem inteira e plana, p. ex. *Notothylas anaporata* Udar & Singh; 2. Talos radiados e grandes, margem lobulada e ondulada, p. ex. *Notothylas dissecta* Steph, e também em *N. nepalensis*; 3. Talos estreitos (como fita), margem inteira e plana, p. ex. *Notothylas khasiana* Udar & Singh, *N. himalayensis* Udar & Singh, *N. levieri* Schiffn. ex Steph, *N. udarii* Singh e *N. pandei* Udar & Chandra (Singh, 2002; Renzaglia *et al.*, 2007, Cobtor, 2005) (Figura 1. A-C).

O hábito das plantas pode variar de planos e prostrados até os lobos do talo eretos formando uma estrutura similar a um “funil” como em *N. himalayensis* Udar & Singh e *N. levieri* Schiffn. ex Steph., a ramos distais expandidos e flabelados como em *N. flabellata* Steph.

b. O Cloroplasto

Os antóceros são grupo irmão de plantas vasculares (Qiu, *et al.* 2006, Renzaglia *et al.* 2008, Chang *et al.* 2011) e o cloroplasto desse grupo pode apresentar características importantes taxonomicas e fisiológicas para o vegetal. O pirenóide é uma área clara que pode ser encontrado nos cloroplastos de 100 das 225 espécies de antóceros (Renzaglia, 2007), incluindo os gêneros *Notothylas*, *Nothoceros* e *Phymatoceros*. O pirenóides apresenta concentração de Rubisco, e isso permite melhorar a eficiência fotossintética (Hanson *et al.* 2002).

Recentes estudos comparativos revelaram a variabilidade no formato do cloroplasto, na quantidade e especialmente na ultraestrutura em antóceros (Duff *et al.* 2007, Renzaglia *et al.* 2007). Os cloroplastos no gênero *Notothylas* são facilmente observados em microscopia óptica, e a visualização dos cloroplastos consiste em dois padrões importantes, essas observações contrastam com trabalhos mais antigos que relatam apenas o cloroplasto único (Renzaglia, 1978; Thomas, *et al.*, 1978):

1. Região de ocorrência e número de pirenóides: Os cloroplastos são claramente distintos com pirenóide na região central em *N. anaporata*, *N. breutelii* e *N. irregularis*, e as espécies *N. dissecta* e *N. nepalensis* não apresentam a região discernível do pirenóide;

2. Aspecto homogêneo ou reticulado do estroma: em *N. dissecta* o estroma é homogêneo, enquanto que em *N. kashyapii* e *N. nepalensis* apresenta o amido elíptico globular compactado, dando a aparência de reticulado. *Notothylas anaporata* apresenta

características similares às mencionada anteriormente, mas esta tem a região do pirenóide bem definido. O cloroplasto em *N. pandei* é diferente dos demais, pois ele tem mais que uma região de pirenóide com a superfície verrucosa.

c. Invólucro

O invólucro é uma camada de células que cobre a cápsula até sua deiscência (Luizi-Ponzo *et al.* 2006). Quase todas as espécies de *Notothylas* tem o desenvolvimento do invólucro horizontal totalmente aderido ao talo e aparentemente confluyente com esse, submarginal a marginais e presos somente nos ângulos agudos-obtusos formados pelos lobos do talo (Singh, 2002). A superfície do involucro pode ser uniformemente lisa ou nodulosa, ou ainda com algumas pregas na região apical (Figura 1. D).

d. Esporângio

No gênero *Notothylas* o esporângio possui a base bulbosa, com uma zona meristemática intermediária e cápsulas usualmente oblongas e cilíndricas notadamente com crescimento definido e limitado, sendo as cápsulas normalmente encontradas com os ápices inteiros (Kenrick & Crane, 1997).

A zona meristemática pode ser bem discreta como em *N. anaporata* e *N. dissecta*, ou muito proeminente como em *N. pfleidereri*, ou ainda condições intermediárias com poucas células pequenas aproximando-se dos exemplos discretos ou com células mais numerosas e maiores (Udar & Singh, 1981; Singh, 2002).

O tamanho do esporângio em *Notothylas* varia de 2 a 4 mm, sendo observados dois padrões relacionados ao tamanho da cápsula, disposição e presença de columela: 1º sem columela, cápsula horizontal e esporângio pequeno (de 1,5 a 3 mm de comprimento)(exceto em *N. kashyapii* onde esta estrutura atinge até três milímetros de comprimento), p. ex. *Notothylas flabelatta*, *N. javanica*; 2º columeladas, cápsula ereta e esporângio maior em comprimento (de 4,5 a 5,5 mm compr.), p. ex. *Notothylas udarii* e *N. dissecta* (Udar & Singh, 1981; Singh, 2002, Villarreal, *et al.* 2010).

e. Cápsula

As cápsulas são ligeiramente elipsoides ou ovais, usualmente chamadas de forma de banana (Gradstein & Costa, 2001). As cápsulas podem apresentar células especializadas que proporcionam a deiscência para liberação dos esporos, sendo dessa maneira claramente bivalves ou, quanto estas células estão ausentes a liberação dos esporos ocorre a partir do

rompimento irregular da cápsula (Udar & Singh, 1981; Singh, 2002).

Para as espécies columeladas a abertura pode ser constituída de 2-3 fileiras de células especializadas, enquanto que as espécies sem columela podem variar de 2-6 células (Asthana & Srivastava, 1992). Estas células de deiscência podem promover ligeira diferença na forma e no diâmetro das cápsulas, as células que constituem a linha de deiscência nas espécies bivalves apresentam coloração mais acentuada e tamanho maior que as demais células (Stieperaere *et al*, 2006) (Figura 1. E-F).

A epiderme da cápsula pode apresentar 2 a 4 camadas de espessura, as células internas são normalmente longo-retangulares e as paredes fortemente espessadas. As células que compõem a camada mais externa são normalmente quadráticas e de paredes finas e hialinas (Singh, 2002). No entanto, podem ser observadas bandas transversais espessadas.

f. Columela

A columela é um tecido estéril na região central da cápsula (Luizi-ponzo *et al*. 2006) e está entre os esporos e pseudo-elatérios. Ela usualmente consiste de 15-17 fileiras verticais de células muito longas que terminam distalmente em uma célula (Figura 2. A-C). A presença ou ausência desta estrutura é muito útil para a taxonomia, inclusive foi utilizada por Singh (1979), Udar & Singh (1981) e Asthana & Srivastava (1992) para a proposição de dois sub-gêneros.

g. Esporos

A maturação dos esporos ocorre do ápice para base, os esporos em diferentes níveis de maturidade mostram uma série de características únicas (cor e ornamentação). A mais comum é a superfície da exina mais ou menos reticulada, devido à deterioração do conteúdo interno. Por isso é necessário o estudo de esporos da mesma zona da cápsula, para garantir o mesmo grau de maturação (Hässel de Menéndez, 1976; Hasegawa, 1979; Udar & Singh, 1980; Singh, 2002).

A morfologia dos esporos e a esporoderme, fornecem a característica taxonômica mais eficiente, eles podem variar muito de espécie para espécie da seguinte maneira:

1. Forma: os esporos são sub piramidais com a face interna apresentando uma cicatriz evidente ou marca trilete, e a face externa pode ou não apresentar uma protuberância (Glime, 2013).

2. Cor: os esporos podem variar de amarelos a verdes quando imaturos, quando maduros, podem se tornar marrons, pretos ou amarelos. Essa característica deve ser levada em

consideração quando observado os esporos maduros.

3. Tamanho: o tamanho dos esporos pode variar de 28-30 a 48-64 μm ;

4. Ornamentação das superfícies externa e interna: Hässel de Menendez (1976) expõe três variações da superfície dos esporos de *Notothylas* extremamente úteis para a diferenciação das espécies: conspicuamente tuberculada, baculada e vermiculada. Ainda podemos acrescentar outra importante característica na superfície externa dos esporos que são as depressões e as protuberâncias na superfície interna (Figure 2. D-G).

h. Pseudo elatérios

Pseudo elatérios são estruturas originadas durante a meiose celular para a produção dos esporos, onde a primeira mitose origina duas células, uma delas forma quatro esporos e a outra é abortada originando um pseudo elatério, que pode ser unicelular ou se dividir novamente e possuir até 5 células (Luizi-ponzo *et al.*, 2006). No gênero *Notothylas* esta estrutura pode ou não estar presente.

As células que formam o pseudo elatério podem ser lineares ou curtas e planas (por ex. *N. anaporata*, *N. breutelii*, *N. orbicularis*), hialinos ou levemente amareladas (exceto em *N. pandei*, os pseudo elatérios são arroxeados) e possuir a parede celular delgada com ou sem espessamentos (Singh, 2002). Esses espessamentos são importantes características para diferenciação das espécies e podem variar desde bandas espessas e proeminentes, levemente espiraladas até inconspícuas com bandas regulares (Figura 2. H-I).

2 Objetivos

- Testar o monofiletismo, com um maior número de amostras no gênero *Notothylas* Sull., incluindo espécies brasileiras;
- Avaliar as características morfológicas que sustentam as espécies dentro do gênero;
- Resolver os conflitos taxonômicos do gênero, através de proposições de sinônimas; busca por tipos nomenclaturais; rever espécies excluídas

3 Materiais e métodos

Respeitando as diretrizes estabelecidas pelo Programa de Pós Graduação, em Biodiversidade Vegetal e Meio Ambiente - Instituto de Botânica/ IBot e devido às facilidades deste modelo, para consequente publicação optou-se pela apresentação da tese em um formato

misto, dividida em formato clássico de tese e em artigos divididos em dois resultados, cada um correspondendo a um manuscrito que será enviado a revistas científicas, e cada um apresenta a caracterização de material e métodos separadamente, organizados da seguinte maneira:

No primeiro resultado apresentamos a revisão das espécies de *Notothylas* para o Brasil, caracterizando o gênero através das características morfológicas do esporófito (cápsula, esporo, columela, pseudo elatério, invólucro) e do gametófito (talo, rizóides, cloroplasto, associação com *Nostoc* cianobactérias). Como principais resultados estão a proposição de um sinônimo e a descrição de duas espécies novas para a ciência.

No segundo resultado é apresentada uma sinopse mundial do gênero *Notothylas*, que foi elaborada através de pesquisa histórica das classificações mais antigas e contemporâneas, através de análise morfológica visamos apresentar um tratamento taxonômico incluindo chaves de identificação, descrições, ilustrações, análise biogeográfica, juntamente com comentários filogenéticos sobre os subgêneros. Como principais resultados estão a sinonímia de subgêneros, a exclusão de algumas espécies duvidosas, os comentários filogenéticos sobre os subgêneros propostos por Ashatana & Srivastava (1992), as ilustrações e o tratamento taxonômico.

4. The genus *Notothylas* (Notothyladaceae, Anthocerotophyta) in Brazil

Leandro A. Almeida¹, Juan Carlos Villarreal A.^{2,3}, and Denilson F. Peralta¹

¹ Instituto de Botânica, Av. Miguel Stéfano, 3687 - CEP 04301902 - São Paulo, SP, Brazil

² Département de Biologie, Université Laval, Québec, Canada

³ Smithsonian Tropical Research Institute, Panama, Panama

³ Corresponding author's e-mail: ednlora@gmail.com, denilsonfperalta@gmail.com,
jcarlos.villarreal@gmail.com

Abstract – The genus *Notothylas* Sull. ex A. Gray, was reviewed for Brazil based on type material, herbarium specimens and recent collections. Five species of the genus are recognized, *N. vitalii* is synonymized with *Notothylas javanica* and two species are new to science: *Notothylas granulosa* Amélio & Peralta and *N. vermiculata* Amélio & Peralta. A diagnostic key, descriptions, illustrations and taxonomic comments are provided.

Key words: Hornworts, new occurrence, new species, taxonomy.

Introduction

Hornworts have been at the center of the discussion of early plant evolution, due to the conflicting placement of the group within the context of land plant phylogeny (Renzaglia et al. 2000; Puttick et al. 2018). A great amount of knowledge of the group has been accumulated in the last 15 years especially in the developmental and genomic aspects (Renzaglia et al. 2008; Li et al. 2014; PNAS. Szövényi, 2016). However, the taxonomy of the group remains poorly understood in many parts of the world, especially in the Neotropics and the African continent. Among the five hornwort families, the family Notothyladaceae has a remarkable place due to the heterogeneity in morphological and molecular characters (Duff et al. 2007; Villarreal & Renner 2015).

The family Notothyladaceae includes four genera worldwide: *Notothylas* Sull. ex A. Gray, *Phaeoceros* Prosk., *Mesoceros* Piippo and *Paraphymatoceros* Hässel (Duff et al. 2007, Söderstrom et al. 2016). Among them, *Notothylas* is remarkable in several morphological

traits such as a small sporophyte (smaller than in any other genus of Anthocerotophyta) typically included within the involucre, absence of stomata (Renzaglia et al. 2017); columella either absent or present; shelf-like arrangement of the pseudoelaters (Renzaglia 1978; Singh 1981) cleistocarpy in several species; chloroplasts (1-2) with or without pyrenoid (Singh 1981) and slightly faster substitution rate (Villarreal & Renner, 2013) probably associated to seasonal life history traits. Unlike all other genera, spore colour can be variable, we could find species with yellow spores (e.g. *Notothylas javanica* (Sande Lac.) Gottsche or dark (brown or black) spores (e.g. *N. breutelii* (Gottsche) Gottsche)).

In addition, there is a great deal of variability in spore ornamentation with the distal face being either vermiculate, baculate, or tuberculate (Singh, 2002; Hässel de Menéndez, 1976). On the proximal face, the presence of a central hollow on each triangular face seems to be delimiting character for several species (e.g. *N. irregularis*, *N. dissecta*). The unique combination of sporophytic characters have been key to propose subgeneric divisions. For example, Asthana & Srivastava (1992) proposed two subgenera, *Notothylas* and *Notothyloides*, based on the presence or absence of dehiscence lines and columella respectively. Schuster (1992) proposed a subgeneric division based on spore colour, surface and a presence of a hollow on a proximal face, section *Notothylas* and *Depressisporae*, respectively. The monophyly of each subgeneric division remain tested using molecular markers. However, the most recent hornwort phylogeny based on 4 markers and limited sampling (9/23 species, Villarreal et al. 2015) suggests that morphology-based subdivisions are not monophyletic.

Notothylas comprises 23 species worldwide (Hasegawa 1979; Villarreal et al. 2010; Chantanaorrapint 2014, 2015). The diversity is unequally distributed with 16 species in continental Asia and Pacific Islands, four species in Africa (*N. decurva*, *N. flabelatta*, *N. indica* and *N. javanica*), one in Europe, two in North America and 5-7 species (*N. breutelii*, *N. dissecta*, *N. javanica*, *N. orbicularis*, *N. vitalii*, and *N. flabellata*) to South America (Villarreal et al. 2010; Hässel de Menéndez et al., 2009; Wigginton, 2002; Hasegawa 1984).

In the American continent the genus has been recently reviewed in North America by Schuster (1992) with a final nomenclatural delimitation by and Stotler & Crandall-Stotler (2005). In Central and South America, the studies have scarce with a few papers documenting local species (Panama, Dauphin et al. 2006), Brazil (Brazil, Gradstein & Costa (2003) and Brazilian Online Flora (FBO 2020). The most detailed systematic treatment was provided by

Gradstein & Costa (2003) in which they recognized three species *Notothylas breutelii*, *N. orbicularis*, and *N. vitalii*.

To fill gaps in the taxonomic knowledge of the genus in South America, we have revised collections from Brazil along with type material to provide a revised taxonomic treatment for the country. We confirmed the presence of five species, two of them new species to science,

Materials and methods

We revised type material from local and international herbaria (G, RB, HERBIT, HUVA, INPA, L, MG, NY, PH, SJRP, SP, UB, UFPE) and three collections from the Brazilian states of Ceará, Maranhão, Rio de Janeiro and São Paulo.

The terminology used in morphological description, habitat, geographic distribution and ecological comments follow Singh & Udar (1981, 2002), Hässel de Menéndez (1976), Renzaglia *et al.* (2008), Hasegawa (1979) and Schuster (1992). Mature spores were placed on double stick adhesive tape affixed on stubs, without the need of a critical point for the metalization and observed using a scanning electron microscopy (SEM).

The observation of the macroscopic structures (thallus and spore colour, gametophyte and sporophyte size, growth form, presence of symbiotic *Nostoc*) was performed under optical microscope and stereomicroscope. Taxonomic and ecological notes, descriptions, key to the species and diagnostic description and illustration for each species are provided. The capsule cells of the wall were measured with a micrometer eyepiece on the optical microscope. The geographic distribution follow the Brazilian geopolitical states (IBGE 2012).

Results and discussion

We recognize five species of *Notothylas* to Brazil (table 1), two species are new to science: *Notothylas granulata* Amélio & Peralta and *Notothylas vermiculata* Amélio & Peralta and we propose the synonymization of *N. vitalii* (Singh 1980) under *N. javanica*. The Brazilian *Notothylas* species are annuals and they are found in open places, often in disturbed areas, ranging from 100 to 1500 m above sea level. The genus is widely distributed in biomes of Caatinga and Savanna, and scattered occurrences in the Amazon and the Atlantic Forest (Figure 3. A).

Taxonomic treatment

Key to species of *Notothylas* in Brazil

1. Mature spores yellow, without hollows in proximal faces - 2
2. Capsule with a dehiscence line; pseudoelaters present - *Notothylas orbicularis*
2. Capsule without a dehiscence line; pseudoelaters absent - *Notothylas javanica*
1. Mature spores yellowish to brown, with or without hollows in proximal faces - 3
3. Inner surface of the spore without a central hollow; spore surface baculate - *Notothylas breutelii*
3. Inner surface of the spore with a central hollow; spore surface vermiculate to tuberculate - 4
4. Spore surface finely tuberculate; proximal hollow tuberculate – *Notothylas granulosa*
4. Spore surface vermiculate; ornamentation confluent with the proximal hollow - *Notothylas vermiculata*

Notothylas breutelii (Gottsche) Gottsche, Bot. Zeitung (Berlin) 16(15): 21, 1858 ≡ *Anthoceros breutelii* Gottsche, Syn. Hepat.: 583. 1846. Type: Ilha Santa Croix, near Friedenthal, *Breutel* s.n. (holotype G00115584!, isotype PC0102910, photo!).

= *Notothylas amazonica* Spruce, Trans. & Proc. Bot. Soc. Edinburgh 15: 578. Type: Andes Peruviani, prope Tarapoto, Spruce s.n. (holotype G00115590, photo!), *syn. fide* Schuster (1992).

= *Notothylas cubana* Steph., Sp. Hepat. 5: 1020, 1917. Type: CUBA, Aguacate, Bayamo, C. Wright s.n. (holotype G00069716!), *syn. fide* Schuster (1992).

Illustration: Figure 3. B-H.

Additional illustrations in Schuster (1992) and Hässel de Menéndez (1976).

Plants flabellate, medium to large size 1–2 cm in diameter. Sporophyte - Involucre dorsal, cylindrical, solitary, 2–3 mm long. Capsule cylindrical, 3 mm long, quadratic to rectangular cell walls 10–26 x 22–64 μm , orange, brown or pale brown, single chloroplast with pyrenoid at center. Monoicous. Spore (36) 47–51 μm dark brown, inner with baculate surface, concave, vermiculate, outer with barely apparent protuberance. Pseudoelater presents 17–27 x 25–63 μm , columella present, capsule opening by a dehiscence line of two rows of cells.

The species has a rather cosmopolitan distribution. In the American continent, it is found throughout tropical America and reaches as far as Louisiana (Pagán 1939a, b; Schuster 1992). In the West Indies, it is known from St. Croix, Virgin Islands (type), Puerto Rico, Cuba, Dominican Republic, and Guadeloupe (Frahm 2012; Lavocat Bernard & Schäfer-Verwimp 2011; Pagán 1939a, b). The species has been reported from man-made places (such as lawns in houses and university campus) Hawaii (Miller 1967) and the Philippines (Hasegawa & Tan 1986).

In Brazil is in the Atlantic Forest and Pantanal biomes, in the states of Bahia, Espírito Santo, Maranhão, Mato Grosso do Sul, Minas Gerais, Pernambuco, and São Paulo (Simonelli & Fraga 2007; Machado 2011; Costa 2012; CNCFlora 2013; FBO 2020, Bojacá et al., 2016), and is recorded here to the first time to Acre, Ceará, Rio de Janeiro.

In Brazil, this specie is found often on moist soil and rocks at 800–1000 m a.s.l., mixed with *Fissidens* spp. and *Targionia hypophylla* L. The distribution of the specimens analyzed show association with man-made habitats (like most *Notothylas* species) and the current distribution may be a reflection of the man dispersal.

Specimens examined: BRAZIL. ACRE: Rio Branco, Zoobotânico park, 28/5/1987, *Vital, D.M.* 14930 (SP). **BAHIA: Salvador**, Campus of Olinda UFBA, 17/9/1984, *Bastos, C.J.P.* s.n. (SP191825), idem, 11/09/1986, *Bastos, C.J.P.* s.n. (ALCB). **CEARÁ: Ubajara**, São Luis site, 29/4/2004, *Oliveira, H.C.* 172 (SP). **ESPÍRITO SANTO: Santa Teresa**, Biological Reserve Augusto Ruschi, 9/10/2002, *Rossini, J.* 60 188 (SP). **MATO GROSSO DO SUL: Bonito**, Córrego Roncador, Ciliary mat, 13/8/2002, *Peralta, D.F.* 1868 (SP). **PERNAMBUCO: Fernando de Noronha**, Alto of Dois Abraços, 2/8/1978, *Vital, D.M.* 8335 (SP), the same, 3/8/1978, *Vital, D.M.* 8341 (SP). **Salgueiro**, 31/7/1978, *Vital, D.M.* 8321 (SP), 25/5/1978, *Vital, D.M.* 8192 (SP). **RIO DE JANEIRO: Ilha Grande**, Freguesia of Santana, 17/7/1966,

Vital, D.M. 930 (SP).

Notothylas javanica (Sande Lac.) Gottsche, Bot. Zeitung (Berlin) 16: 20. 1858 $\equiv$ *Blasia javanica* Sande Lac., Syn. hepat. Jav.: 94. 1856. Type: Indonesia. Java, *D.G. Holle s.n.* (holotype L0061010!).

= *Notothylas vitalii* Udar & D.K. Singh, Misc. Bryol. Lichenol. 8: 173. f. 1. 1980. Type: Brazil, Mato Grosso do Sul, munic. de Miranda, Seção de Guaicurus (20°04'S, 56°46'W), in the bottom of a dried lake, ca 8km N from the main house of Fazenda Bodoquena, 11-VI-1973, *D.M. Vital* 2367 (holotype SP88126!, paratypes SP88125!), *syn. nov.*

Illustration: Figure 4. A-E.

Additional illustration and description in Singh (1980) and Chantanaorrapint (2015).

Plants in rosettes, 1–1,5 cm in diameter. Sporophyte - Involucres marginal next to the lobes of the thallus, capsule completely enveloped, 1–1,5 mm long, irregularly arranged epidermal cell wall, rectangular to quadratic 14–45 x 32–75 μ m, with moderately thick wall. Chloroplast 1–2 per cell with a central pyrenoid, but sometimes not discernible. Monoicous. Spore yellow 50–62 μ m, delicately vermiculated surface, with protuberance in dorsal view, pseudoelater absent, columella absent, or not well-developed. Capsule opening by irregular rupture (cleistocarp) without a dehiscence line.

The species has a cosmopolitan distribution, it has been reported from Africa (Wigginton, 2002) China (Hasegawa 1979; Piippo 1990; Lin 2000), Congo, Indonesia (Java), Japan, Panama, Philippines, Thailand (Hasegawa 1979; Dauphin *et al.* 2006; Stieperaere & Matcham 2007; Lai *et al.* 2008).

In Brazil, it has been recorded from the Caatinga and Savanna Biomes, in the states of Amazonas, Ceará, Goiás, Mato Grosso, Mato Grosso do Sul, Maranhão, and Pernambuco (Udar & Singh 1989; Gradstein & Costa 2003; FBO 2020; Bojacá *et al.*, 2016). *Notothylas javanica* is recorded here to the first time from Acre, Bahia, and São Paulo. It is often founded often on moist soil in flower beds and farms, on shaded sandy soil by roads, usually grows on

more or less disturbed places from 100–1500 m a.s.l., often associated with *Fissidens* spp.

Notothylas vitalii was described as endemic to Brazil (Singh 1980). One of the main characters were the presence of a transverse dehiscence line in the capsule, vermiculate spores, lack of columella and pseudolaters. Our study of type material of both species show that they overlap in sporophytic characters, such as the absence of the dehiscence line, columella and pseudoelaters. Additionally, the yellow vermiculate spores are nearly identical in both species. The absence of a dehiscence line is an important character to delimitate some species, such as the Japanese *N. tempera* Hasegawa and the Chinese *N. yunannensis* T. Peng & R. L. Zhu. *Notothylas vitalii* was described as a noncollumelate species, the presence or absence of columella seems to be a critical character to circumscribe species but the taxonomic value at the species level needs to be reconsidered (Schuster, 1992; Hasegawa, 1978). For the moment, we suggest to synonymize both species, but further molecular work is essential to define whether *N. javanica* is a single widespread species or it represents a cluster of cryptic species.

Specimens examined: **BRAZIL. ACRE: Rio Branco**, Socorros site, 31/5/1987, *Vital, D.M.* 14996 (SP), the same, 1/6/1987, *Vital, D.M.* 15025, 15032, 15037 (SP), *idem*, 26/5/1987, *Vital, D.M.* 256928 (SP). **BAHIA: Feira de Santana**, 23/10/1990, *Yano, O.* 15058 (SP), the same, Campus da Universidade Estadual of Feira de Santana, 29/6/2009, *Peralta, D.F.* 449122 (SP). **Ilhéus**, CEPEC (Centro de Pesquisas do Cacau), 15/7/1991, *Vital, D.M.* 20167 (SP). **MARANHÃO: Caxias**, Auto do Estevão, 17/6/2007, *Brito, E.S.* 261 (SP). **Carolina**, Nacional Park da Chapada das Mesas, Cachoeira do Alegre, 3/22/2017, *Amélio, L.A.* 318, 319, 320, 321, 322, 323, 324, 325, 326, 327, 328, 329 (SP). **Zé Doca**, Povoado Quinto Braço, 4/15/2017, *Oliveira, R.R.*, 667 (SP). **Imperatriz**, 15/2/1974, *Vital, D.M.* 2971 (SP). **São João de Soter**, 19/04/2015, *Vieira, H.* 160 (SP). **MATO GROSSO DO SUL: Bonito**, Rio Mimoso, riparian forest, 4/6/2002, *Peralta, D.F.* 1702 (SP). **Miranda**, 6/6/1973, *Vital, D.M.* 2325 (SP), *idem*, 9/6/1973, *Vital, D.M.* 2355 (SP), *idem*, 11/6/1973, *Vital, D.M.* 2367 (SP), *idem*, Córrego Coqueiro, riparian forest, 14/8/2002, *Peralta, D.F.* 1973, 1988 (SP). **PERNAMBUCO: Cabo**, Gurjau station, 14/1/1984, *Yano, O.* 9145, 9193 (SP). **Fernando de Noronha**, Ca. 2 Km NE do Alto dos Dois Abraços, 31/7/1978, *Vital, D.M.* 133193, 133194 (SP). 7/14/2016, *Duckett, J., s.n.* (SP). Trilha do Capim Açú, 14/7/2016, *Costa, D. P. et al.* 6413, 6418, 6419, 6423, 6427, 6434, 6437 (RB). Praça do Flamboyant 13/7/2016, *Costa, D. P. et al.* 6329, 6330 (RB). Trilha dos mirantes dos Golfinhos 14/7/2016, *Costa, D. P. et al.* 6399 (RB). **Recife**,

Cidade Universitaria, 24/7/1984, *Leão Barros, I.C. s.n.* (SP, UFP), the same, 3/8/1994, *Yano, O. 23059* (SP), the same, 16/5/1997, *Yano, O. 24808* (SP), the same, 10/9/1984, *Yano, O. 9068* (SP), the same, 4/9/1984, *Yano, O. 9036* (SP), the same, Mata de Dois Irmãos, 5/8/1998, *Yano, O. 25418* (SP), the same, Campus da Universidade, 5/8/1998, *Yano, O. 25422* (SP) the same 04/8/1998, *Costa, D. P. 3362* (RB). **SÃO PAULO: Cajuru**, 23/3/1982, *Vital, D.M. 10370* (SP). **Juquiá**, 16/7/1977, *Vital, D.M. 7175* (SP).

Notothyas orbicularis (Schwein.) Sull. ex A.Gray., J. Sci. Arts, ser. 2 1: 74. 1845 [1846]≡ *Carpolipum orbiculare* (Schwein.) Nees, Syn. Hepat. (fasc. 4): 591. 1846 ≡ *Carpobolus orbicularis* (Schwein.) Schwein., J. Acad. Nat. Sci. Phi. 2: 366. 1822 ≡ *Targionia orbicularis* Schwein., Spec. Fl. Amer. Sept. Crypt.: 23. 1821. **Type:** U.S.A., North Carolina, Forsyth, Salem, on moist earth, *Schweinitz s.n.* (holotype PH00003638!).

= *Notothyas angolensis* Steph., Cat. Afr. Pl. 2(2): 320. 1901. Type: Angola, Pungo Andongo, *F. Welwitsch s.n.* (holotype G00066857!), syn. fide. Jones & Harrington (1983), and Schuster (1992)

= *Notothyas melanospora* Sull., Amer. J. Sci. Arts, ser. 2 1: 75. 1845 [1846]. Type: United States, Ohio, Franklin Co., s.col. s.n. (holotype NY00231521!), syn. fide Schuster (1992).

Illustration: Figure 4. F-L.

Additional illustrations and descriptions in Hasegawa (1979), Schuster (1992), Chantanaorrapint (2015), Cargill (2016) and Kobtor (2018).

Plants in rosettes 2 cm in diameter prostrate. Sporophyte – Involucre covering the entire immature capsule. Capsule completely enveloped 1.6 – 2 mm, irregularly arranged epidermal cell wall, rectangular to quadratic 20-23 X 35-45 µm, with thick cell walls. Chloroplast 1 (–3) per cell with pyrenoid present, but sometimes it is difficult to discern. Spore 35–42 (–45) µm yellow slightly brownish (size), with vermiculate surface, trilete mark present, without protuberance in dorsal view. Pseudoelaters presents, 15–32 x 28–62, pale

yellow to brown. Columella well developed and persistent, with irregular helicoidal thickening bands. Capsule opening per dehiscence line of two, rarely 3 rows of cells.

The species has a cosmopolitan distribution. *Notothylas orbicularis* has been reported from America, Africa, Europe and Asia (Schuster 1992; Lai *et al.* 2008; Peng & Zhu 2014). In Brazil, it has been collected in the Caatinga and Savanna Biomes in the states of Bahia, Maranhão, Mato Grosso, Pernambuco (FBO 2020, Bojacá *et al.*, 2016), and is recorded here to the first time to Goiás e Piauí. It is often founded often on disturbed soil along walking trails between 50 and 700 m a.s.l., mixed with *Targionia* spp. and *Fissidens* spp. The molecular delimitation of *N. orbicularis* and *N. javanica* is awaiting for further molecular work.

Specimens examined: **BAHIA:** Cruz das Almas, 24/6/2004, Peralta, D.F. 2466 (SP). Ilhéus, CEPEC (Centro de Pesquisas do Cacau), 15/7/1991, Vital, D.M. 20168 (SP). **GOIÁS:** Itaberaí, 27/1/1973, Vital, D.M. 2236 (SP). Mossâmedes, Estação Biológica Serra Dourada, 21/3/1990, Yano, O. 14164 (SP). Santa Tereza de Goiás, 18/2/1974, Vital, D.M. 3028 (SP). **Maranhão:** Zé Doca, Aldeia dos Guajajaras, 8/30/2017, Oliveira, R.R. & Oliveira, R.F., 79 (SP). **MATO GROSSO:** Cuiabá, 20/6/1981, Vital, D.M. 134271 (SP). **PERNAMBUCO:** Bituri Grande, Brejo da Madre de Deus, 10/8/1998, Yano, O. 25502 (SP). **Piauí:** Ubajara, São Luís site, Oliveira, H.C. 8, 146 (HUVA).

Notothylas granulata Amélio & Peralta, D.F. **Type:** Brazil. Pernambuco: Fernando de Noronha 3/8/1978, Vital, D.M. 8341 (holotype 133199). *sp. nov.*

Illustration: Figure 5. A-K. A. Rehydrated plant. B. Distal cells of the capsule. C. Light microscopy of the proximal spore surface. D. Light microscopy of the distal spore surface. E-G. Pseudoelaters. H. SEM of the proximal spore surface. I. Detail of the ornamentation of the hollow on the proximal face. J. SEM view of the distal face of the spore. K. Detail of the ornamentation on the distal surface.

Diagnosis: *Notothylas granulata* is close to *N. dissecta* Steph. and differs in the distal surface of the spore without a central hump-like projection, and the proximal surface the hollows are not radiating by the tubercles, the thallus growth form (fasciculated in *N. granulata* and caespitose rosettes in *N. pandei*), and the pseudoelaters without spiral bands.

Thallus light green to yellow, with fasciculated margin, 1.5–2.5 cm in diameter, adhered to the substrate, rosulate to flabellate, without cavities, dorsally flattened in cross-section 5–8 cells, smooth surface; margin lobed but with narrow lobes, with truncated apex. Cells in the dorsal region of the epidermis quadratic or hexagonal, rhomboidal, 30–60 x 20–50 µm with one chloroplast and pyrenoid evident. *Nostoc* irregularly disposed around stem. **Rhizoids** hyaline lightly brown, with smooth to wrinkled walls. **Monoicous. Androecia** scattered, antheridia 2–4 per cavity, globose to subglobose. **Involucre**, carrying just one capsule disposed horizontally prostrated or slightly upward to the thallus, cylindrical or conical, slightly curved, 1.5–2.5 mm long and 0.2–0.5 mm in diameter. **Capsule** with longitudinal dehiscence, with a row of differentiated cell; rectangular elongated cells, quadratic, rhomboidal, brown reddish to ochre, with wide wall, 40–120 x 15–25 µm. Oblong, quadratic, and rhomboidal, dark yellow to mustard capsule cells; arranged from longest to shortest, from the margin to the center. **Columella** well developed, persistent with thickenings on the cell surface. **Spores** (25) 30–36 µm, brownish, unicellular, tetrahedral and well-defined and smooth trilete mark, shallow depressions in the center of each triangular face. Granulate spore ornamentation, 0.5–1 µm granules in the outer surface; concave spore with bulge in the outer central surface.

The epithet “*granulata*” is referring to the surface of the spore, which is taxonomically referred as granulate, *Notothylas granulata* has granules very small on spore surface.

Notothylas granulata is morphologically similar to *N. dissecta* Steph., a Central American species with a disjunct distribution in India. They differ in the absence of a hump-like projection on the distal spore face, the proximal surface without tubercles radiating from the central hollow and a fasciculate gametophyte. The new species is similar to *N. pandei* Udar & Chandra, an Indian species with tuberculate spores (Asthana & Srivastava, 1992) but differ by the presence of purple pseudoelaters and the spores without a hump like projection on the distal surface. *Notothylas granulata* resembles the Southern African *N. flabellata*. They share the granules in the spore ornamentation, but they differ in the presence of a columella and pseudoelaters, the outer surface of spore with 2–3 globular projections. *N. granulata* lacks projections on the distal spore surface.

The specimen was found growing with mosses such as *Fissidens veracruzensis* Pursell and *F. reticulosus* (Müll. Hal.) Mitt., in wet ravines, on disturbed soil ca. 100–300 m above sea level. *Notothylas granulata* is known only from the type locality; however it may also occur in other areas in northern and North-western Brazil and the Neotropics with similar climatic condition.

Notothylas vermiculata Amélio & Peralta, D.F. Type: BRAZIL. **Ilhéus**, Área do CEPEC (Centro de Pesquisas do Cacau), 15/7/1991, Vital, D.M. s.n. (SP404132), *sp. nov.*

Figure 6. A–J. A. Rehydrated plant. B. Distal cells of the capsule. C. Light microscopy of the proximal spore surface. D. Light microscopy of the distal spore surface. E–F. Pseudoelaters. G. SEM of the proximal spore surface. H. Detail of the ornamentation of the hollow on the proximal face. I. SEM view of the distal ace of the spore. J. Detail of the ornamentation on the distal surface.

Diagnosis: *Notothylas vermiculata* is similar to *N. irregularis* Chantanaorr. It differs in the outer side of the spore wich lacks a hump-like projection and the coarsely vermiculate surface.

Thallus greenish yellow to light green with fasciculated margin, 3–5 cm in diameter, prostrate or strongly attached to the substrate with few and short (brief) branches, without cavities, dorsally flattened in cross-section 5–8 cells, smooth surface; margin lobed but with narrow lobes, with truncated apex. Cells in the dorsal region of the epidermis quadratic or hexagonal, rhomboidal, 30–60 x 20–50 µm, with one chloroplast and pirenoid evident. *Nostoc* irregularly disposed around the thallus. **Hyaline** rhizoids lightly brown, with smooth or wrinkled walls. **Monoicous**. **Androecia** scattered and antheridia 2-3 per cavity, subglobose, 90 – 106 x 86 – 102 µm. **Involucres** solitary, carrying a capsule prostate horizontally or slightly upwards the stem, cylindrical or tapered, longitudinally plicate or lamellate (not lobed). **Capsule** slightly curved, 1.0–1.2 mm long, 0.2–0.5 mm diameter, fully when covered by involucre with longitudinal dehiscence, by two different cell rows. The outer row with rectangular cells, quadratic, brown color rhomboidal reddish to orange, 30–50 x 15–30 µm; Internal row has more elongated and thinner cells, 35–70 x 10–20 µm quadratic, rhomboidal

light yellow color hyaline, the same color as the remaining cells in the capsule. Cells of the capsule, rectangular 40–60 x 10–20 μm pale yellow arranged irregularly. **Columella** well developed, persistent with outer thickenings. **Spores** yellow to pale brown, unicellular, tetrahedral, marked with trilete, 27–38 μm diameter; triangular view of the inner surface, sub pyramidal, forming three subunits, deep depressions in the center of each subunit; vermiculate surface except the scar, as well as the marginal region of the spore. Vermiculate outer surface with broad spokes arranged under depression; Concave spores bulging in the center of the distal region; strongly vermiculate in the proximal and distal face and outer surface.

The epithet “*vermiculata*” is referring to the ornamentation of the surface of the spore (inner and outer), which is taxonomically referred to as coarsely vermicular.

Notothylas vermiculata is morphologically close to *Notothylas irregularis* Chantanaorr. and *Notothylas yunannensis* T. Peng & R.L.Zhu.. These species are found in Thailand and China, respectively. These species have in common with *N. vermiculata* the yellow brownish vermiculate spores with a small hollow on each subunit of proximal face, and sub quadrate to rectangular, and the irregularly arranged epidermal capsule cells. *Notothylas vermiculata* differs from *N. irregularis* by the the strongly vermiculate proximal face, directly to the hollow and the presence of a distal hump-like structure. *Notothylas vermiculata* differs from *N. yunannensis* by the presence of a dehiscence line of the 2–3(–4) cells and pseudoelaters. A phylogenetic study may provide clues on the phylogenetic placement of the species and the relationship with other Asiatic taxa.

The type specimen was found growing on disturbed soil along a walking trail ca. 300–500 m above sea level, over a cashew tree. *Notothylas vermiculata* is known only from the type locality; however it may also occur in other areas in northern and North-western Brazil with similar climatic condition.

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4.2. A world synopsis of the genus *Notothylas* Sull.

(*Notothyladaceae*, *Anthocerotophyta*) with phylogenetic comments

Abstract

This study presents a synopsis of the genus *Notothylas* to the world, investigating the division into two subgenera following Asthana & Srivastava (1992), with molecular tools. The genus *Notothylas* represents almost a quarter percent of the whole *Anthocerotophyta*, and it is characterized mainly by the reduced sporophyte generation without the presence of stomata and with a big chloroplasts with or not pyrenoid. We analyse fresh and herbarium samples as well as original description and online sources. The results was present as a dendrogram of morphological and phylogram to Maxima Parsimony, Maxima Likelihood and Maxima Bayesian. We synonymize the subgenus, recognize 20 species (including the synonymization of *N. vitalii* with *N. javanica*, and *N. levieri* with *N. flabelata* and excluding *N. chaudhurii*, *N. verdoornii*, *N. paroicus*) and provide comments of taxonomy and biogeography, and resolution of taxonomic conflicts as .

Keywords: Subgenera, *incertae cedis*, molecular tools, bryophytes taxonomy, hornworts.

Introduction

The bryophytes are a widespread and highly diversified group of plants, managing to colonize all terrestrial ecosystems. The Bryophytes *latu sensu* constitutes the second largest group of terrestrial plants (Buck & Goffinet 2000), with less species than the group of Angiosperms. They are paraphyletic with three monophyletic divisions: Anthocerotophyta (hornwortss), Marchantiophyta (liverworts) and Bryophyta (mosses) (Buck & Goffinet 2000, Puttick *et al.* 2018).

Phylogenetically the Anthocerotophyta is the oldest group of terrestrial plants and the closest one to the vascular plants (Villarreal *et al.* 2015). This group has approximately 225 species around of the world (Söderström *et al.* 2016). They include plants that have one to eight big chloroplasts per epidermal cell, as well as colonies of the bacteria *Nostoc* sp. immersed at thallus, the long cylindrical bivalve capsule with foot completely immersed in the thallus, stomata on the sporophyte and asynchronous development of the spores (Gradstein *et al.* 2001).

The relationships among the plant lineages, and land plants diversified into these lineages is a question, which remains unresolved (Renzaglia *et al.* 2008). Hornworts are key lineage in raveling the early diversification of land plants (Qiu *et al.* 2006; Renzaglia *et al.* 2009; Shaw *et al.* 2011, Puttick *et al.* 2018).

Actually, the division is distributed in 5 families and 12 genera (Söderström *et al.* 2016, Renzaglia *et al.* 2009). The family Notothyladaceae includes four genera worldwide: *Notothylas* Sull., *Mesoceros* Piippo, *Paraphymatoceros* Hässel, *Phaeoceros* Prosk. This family need a review, to prove the taxonomic value (Söderström *et al.* 2016).

Notothylas comprises thirty-two species described around the world; these species range the globe in the tropical area (Södertröm et al., 2016). However, there are endemic species with narrow phytogeographic occurrence and cosmopolite ones as *Notothylas orbicularis* (Schwein.) Sull., *N. breutelii* (Gott.) Gott., *N. flabellata* Steph., *N. dissecta* Steph., *N. javanica* (Sande Lac.) Gott. and *N. temperata* J. Hasegawa (Hassel de Menendez, 1976; Hasegawa, 1979; Schuster, 1992; Singh, 1979, 1994, 1994a).

Notothylas is characterized mainly for: thallus shape (rosette, flabellate or linear), presence or absence of the columella, dehiscence line (presente or absente), pseudoelaters, and the differentiation of spores surface (baculate, vermiculate and tuberculate), colour and ornamentation (Renzaglia *et al.*, 2008, Hässel de Menendez, 1976). The sporophyte is smaller than any other genus of hornworts (Singh *et al.*, 2001, 2002). With main tropical distribution and with some species reaching the subtropical region (Villarreal *et al.*, 2012).

Asthana and Srivastava (1992) propose two subgenera when they study the Indian species of the genus: *Notothylas* subgenus *Notothylas*, and subgenus *Notothyloides*, where it includes the persistence or absence of the columella as distinctive character. However, these subgenera classification include only species occurring in India. Consequently, there are several species without position between these subgenera and currently classified as *incertae sides*.

Notothylas is recognize as monophyletic genus (Duff *et al.* 2004, 2007) but has never been studied isolated with molecular tools. Renzaglia *et al.* (2007) points out the relationship with *Phaeoceros* but did not include the subgeneric classification in the discussion. This study, analyses all names included in the genus *Notothylas* of the world, and testing the subgenera *Notothylas* and *Notothyloides* classification proposed by Asthana & Srivastava (1992) with molecular tools.

Materials and Methods

Morphological delimitation

The circumscription of family and genus used in this study followed Frey & Stech 2009. The genus *Notothylas* were analyzed, the species delimitation were based mainly on the type, fresh and herbarium specimens.

The diagnostic key was elaborated using the web site <http://xper.com>, and illustrations were prepared by means of software PhotoShop. The names source from the websites Tropicos and JStor, the publications of Singh (2002), Söderstrong (2016), as well as the prologues. The specimens types examined were located through the prologues, JStor Global Plants website, Species link, being analyzed 11 types (2 NY, 1 L, 2 PH, 5 G, 2 SP), and 92 samples (77 SP, 15 RJB).

The samples were analyzed in the Laboratory of the Bryology in Institute of Botany (IBt). The dehydrated samples under glass slide with distilled water and humectol and dissected with styli for direct observation or through cross-sections of diagnostic morphological structures. When necessary the slides were Permanent slides with Hoyer's solution (Anderson 1954) and with Kaiser's glycerine gelatine (Zander 2003) were prepared.

The observation of the macroscopic structures (coloration, size, type of branch of the gametophyte, form of growth, arrangement and location of *Nostoc*) was performed under optical microscope and stereomicroscope. The typical specimen illustration with the help of a camera coupled under the microscope. The structures were measured through a micrometer

eyepiece on the optical microscope. The variations presented by plant size (width, length) were arbitrated following Vaz-Imbassahy *et al.* (2009).

The spores were characterized in Scanning Electron Microscopy by metallization without critical point. The type materials were photographed and analyzed with color scale, and the comparison of the description, characterization of some species, as well as the nomenclature used in morphology, habitat, geographic distribution and ecology follows Singh et Udar (1981, 2002), Villarreal (2017), Hasegawa (1979), Schuster (1992), and Hassel de Menendez (1976).

The phytogeographic distribution followed the information contained in the labels of the exsiccatas and collections made. The classification of the vegetation follows the rules of the Technical Manual of Brazilian Vegetation (IBGE, 2012). In order to cover other plant formations and to complete sampling gaps, collections were made in the Northwest Brazilian in the National Park of the Chapadas das Mesas in Maranhão, as well as collections in the State of Ceará, since previous records report the occurrence of *Notothylas* in areas of Atlantic Forest and Cerrado.

From the characteristics of the samples analyzed we built a table with the relevant characters in order to perform a cladistic analysis with the software PAST. The absence or presence was described as “0 - 1” respectively; to qualitative characters we established groups as “1, 2 and 3”, and to ordinal characters like number of cells in the dehiscence line we used “0, 2, 3, 4”. In total ten characters were analysed in clustering, followed by likelihood analyses and it resulted in a consensus tree of the similarity of characters (Atwood, 2007).

Molecular study

Sampling - 22 samples were selected to genus *Notothylas* and 9 of the outgroup *Phaeoceros* Prosk. The SP herbarium has recent samples to molecular analysis, and the GenBank has a range of sequences than was used to this analyses.

Extraction of the DNA and amplification - The genomic DNA was extracted from the fresh material that was collected on the northeast of Brazil and of the material herbarium when it was allowed. The species selected to extraction were cleaned on water with the aid of a brush aiming at the removal of the earth, remains of substrates and other species that happen to have developed associated, finally, only the gametophytes were separated for use, to avoid the mix of ploidy.

To obtain news sequences the DNA was extracted by the mini CTAB method (Doyle

& Doyle 1987, 1990) with protocols modified by Câmara (2006, 2010). After extraction, the DNA was amplified through the Polymerase Chain Reaction (PCR), using three markers for the chloroplast region *trnL* (Quandt & Stech, 2004), Rubisco small subunit (*rbcS*) (Villarreal unpub.) and *rbcL* (Duff et al. 2004)(Annex III). The sequences available in the genbank were also used to increase the data matrix.

To amplify PCR mixes were prepared by multiplying the per sample quantities by the number of samples required, then aliquots distributed. Partial *rbcL* was used in the region for amplification, this area has approximately 1300 bases pair. The PCR (Câmara 2006, 2010) was used consisting of, per sample, 26.5µl dH₂O, 4µl dNTPs, 5µl 10x Buffer, 5µl of MgCl₂ at 50nM concentration, 2.5µl of each primer at 10nM, 4µl of Bovine serum albumin (BSA) and 0.5 Taq. 2µl of DNA extract template is then added to 48µl of the PCR mix now in PCR strip tubes.

A PCR profile of an initial 95°C to desature for 1 minute followed by 35 cycles of 95°C denaturation step for 15 seconds. To ensure a more efficient amplification, the samples were submitted of 55°C for 15 seconds and 72°C for 30 seconds for fragment elongation, and then a final 7 minutes at 72°C. This were all carried out in a DNA PCR machine Pro Flex-PCR System, which at last was maintained to conservation -4°C.

Gel Electrophoresis - It was run gels made with 1g of agarose dissolved in 100ml of 1xTBE (Tris/Borate/EDTA) buffer and left to cool and set for 30 minutes. To coloration, 2µl of ethidium bromide was utilized, to see DNA bands on UV excitation. With the gels solid were placed into electrophoresis tanks and then submerged in 1xTBE running buffer.

To prepare the samples in the gel, 2µl were added for each sample PCR product with 3µl of the glycerol/bromophenol blue. Electrophoresis tank were connected in a power pack and the gel ran for 30 minutes at 100 volts to allow for sufficient DNA migration. The gel, in its tray, were transferred to an ultraviolet chamber. This connected to a desktop computer running the software UVP DNA that is able to capture, optimise and save images of the gel. Isolated bands lacking laddering or smearing and of an expected product size indicate a PCR product with good quality sequence data, brighter bands were the desirable product.

Phylogenetic delimitation - The PCR products were sequenced directly from MACROGEN®. The sequences was compared with those obtained from GenBank using the BLAST tool (Altschul et al., 1990). GenBank (<http://www.ncbi.nlm.nih.gov/genbank>) has available sequences of nine species of *Notothylas* (*N. breutelii* (Gottsche) Gottsche, *N.*

dissecta Steph., *N. himalayensis* Udar & D.K. Singh, *N. indica* Kashyap, *N. javanica* (Sande Lac.) Gottsche, *N. levieri* Schiffn. ex Steph, *N. orbicularis* (Schwein.) Sull., *N. pandei* Udar & V. Chandra, *N. vitalii* Udar & Singh).

Sequence alignment and editing with the BioEdit software (Hall, 2005). The analysis of maximum parsimony (MP) and Likelihood were performed with MEGA software (Kumar, Stecher, and Tamura 2015). The maximum parsimony and likelihood searches were based on the heuristic search, 1,000 (random-addition-sequence replicates), branching (tree bisection-reconnection), MulTrees on, and collapse zero-length branches off. The characters were treated as balanced and not ordered. When more than one parsimony tree is found, these were summarized as just one tree of strict consensus. Nonparametric bootstrap values (Felsenstein, 1985) were generated as heuristic searches with 1,000 replicates, each with ten random replicates. Rearrangements were restricted to 1,000,000 per repetition. Bootstrap percent value (BPV) ≥ 70 were considered as good support (Hillis & Bull 1993). The strict consensus trees of the non-parametric bootstrap analysis were visually analyzed to identify conflicting nodes supported by at least 70% (Mason-Gammer & Kellog 1996). Bayesian inferences were made in Mr. Bayes software v.3.2.6 (Ronquist & Huelsenbeck 2003) the support for the nodes was calculated by means of subsequent probabilities. The values of the posterior probabilities vary between 0 and 1. Such analyses were processed in four runs, each with two MCMC chains (Markov Chain Monte Carlo) run for 5,000,000 generations, sampled every 1000 generations and in parallel runs. The first 25% of the trees were burned. The consensus tree was constructed. The characteristics of MP, ML and Bayesian inferences of the *rbcL* region were summarized in Annex 1.

Tracer 1.5 software (Rambaut & Drummond 2013) was used to determine when tree sampling was stabilized.

Results and Discussion

Morphometric results

The cluster analysis and the principal components analysis were performed on the data matrix, using a measure of distance, the Cluster analysis identifies groups based on their similarities by minimizing within-group variation and maximizing between-group variation. Both analyses showed correlations among the 10 morphological characters and were used to groupings of the 21 species.

The result was a dendrogram, the nominal category of the software identifies as base

to differentiation of the similarity, and subsequently the categories ordinal and group, as base to similarity between the species. The main components analysis of the data matrix (Annex IV) revealed that characters that can be identified as apomorphic, it were used in this analyse as main components to similarity and characters that together can bring species closer. The Fig. 7 present the similarities between the species following the same pattern of distribution of the value, and characters as previously described.

This result do not support the subgenera described by Asthana & Srivastava.

Molecular Evidence and Phylogeny comments

Our data set includes about 1,300 total characters with 624 variable characters that were phylogenetic analysed using rbcL (Annex V) chloroplast data supporting the placement of *Notothylas* as a monophyletic taxon as was represented in Qiu 2006, Renzaglia *et al.* 2008, Frey and Steck 2009. The data cluster *Notothylas* in a highly supported clade (100% bootstrap value). The use of Genbank dataset, promotes a better perspective of region and differentiation of species.

The analysis by MP (Fig. 8), result in a cladogram that present a good support to genus, the monophyletic (99), and points some groups of the specimens which probably were from island, isolated geographically, the same plants does not represent morphological differentiation. The cladogram of ML (Fig. 8) and BY a filogram (Fig. 9) shows not very different of the MP, points, and the same differentiation, with the presence of some groups (61, 61, 88 values), which this specimens provide from island, like MP analyses, the differentiation with the outgroup is clear.

The Checklist of Hornworts and liverworts of the world (Söderman et al 2016) brought the genus *Notothylas*, subdivided in three parts. The subgenus *Notothylas*, the subgenus *Notothyloides* and the *incertae sedis*, which includes most part of the clade. The results of this study confirms, from both molecular and morphology, the *Notothylas* status as a genus without the division on two subgenera, the base to include this taxonomic differentiation was established just by morphological characters, which showed that it do not have support to maintain the status.

Gottsche (1858) measures the taxonomic importance of the characteristics of the spore color and surface ornamentation, and describes two subgroups (Eu-*Notothylas* and *Acanthonotothylas*). However, the Gottsche subdivisions was made with few species described, and after news species discovery, this classification lose the perspective. Schuster (1992) made a subgeneric division with the spores colors and presence or absence of hollow

in the proximal face (Section *Notothylas* and *Depressisporae*). The hollows in the spore maybe to be a good characters to subsections, the presence or absence are not associated with another structure and the presence change the spore surface. This study are not propose a new subgeneric division but the close this to just one, until equipped with news molecular results may separate the species not just about morphologic structures.

Molecular tools changes to *Notothylas*

The molecular evidences to hornworts started a few years ago but without a protocol appropriated to study on perspective of the genus. The study with *Notothylas* was not easy mainly to determine the protocol to use. The earlies studies present one regions tested (rbcL), which represented in cladograma.

The extraction using the method of Doyle and Doyle (1990) with modification of Câmara (2006) achieved the expected performance, and it can be a good method to DNA extraction. Although the use of kits may provide better results. To PCR protocols, and the use of the new materials, like Taqs, primers and others may promote the more verified to dataset. To the primers rbcS and trnL not obtain results with the use of established protocols, which to have been related to reagent incongruence regarding the genus or the inefficiency of the primer used. The circumscription to genus are not complete, and need studies to determine if *Notothylas* had subdivisions.

The analyses based on the marker rbcL, although this is a conserved region. The genus *Phaeoceros* Prosk was used as an out group that is included in the *Notothyladaceae*. The analyses of the subgenera by molecular tools to rbcL region do not shows differentiation of the specimens into two subgenera, the range of structures did not reflect this analysis.

Despite this result, it is necessary the use of more markers, and the use of others regions such as nucleus and mitochondria. Alliances to achieve this goal must be made, since the genus is not endemic.

Morphological delimitation

This study included twenty species of *Notothylas*, with highlight of two species that are new to science, and three excluded names. Ten types and 92 specimens from G, RB, HERBIT, HUVA, INPA, L, MG, NY, PH, SJRP, SP, UB, UFPE.

To identify *Notothylas* species with the key provide here is necessary to observe the gametophyte with sporophytes, the shape of the thallus (rosulate, flabellate or linear) and the dispersion of the *Nostoc* colonies. The sporophyte characters to observe includes the capsule

(columella and dehiscence line), and the spores (color and ornamentation).

Our morphological and molecular analyzes do not support the division of *Notothylas* in two subgenera, for this reason we propose the synonymization of subgenus *Notothyladoides* with the subgenus *Notothylas*.

Taxonomic treatment

Notothylas Sull. ex A. Gray, Musci Alleg. n°.290. 1845 & Mem. Amer. Acad. Arts Sci. N.S.3: 65. 1848. Type: *Notothylas orbicularis* (Schwein.) Sull. ex A.Gray., J. Sci. Arts, ser. 2 1: 74. 1845 [1846] ≡ *Targionia orbicularis* Schwein., Spec. Fl. Amer. Sept. Crypt.: 23. 1821. ≡ *Carpobolus* Schwein., Jour. Acad. Nat. Sci. Philad. 2: 361. 1822. ≡ *Carpolipum* Nees in G. L. & N., Syn. Hep. 591. 1846. ≡ *Chamaeceros* Milde, Nova Acta 1856. ≡ *Blasia* Sande Lac., Syn. Hep. Javanicarum 1856. Type: U.S.A., North Carolina, Forsyth, Salem, on moist earth, *Schweinitz s.n.* (PH00003638!).

= *Acanthonotothylas* Gottsche, Übersicht und Kritische Würdigung der seit dem erscheinen der Synopsis Hepaticarum bekannt gewordenen Leistungen in der Hepaticologie, 1858. Type: *Notothylas breutelii* (Gottsche) Gottsche, Bot. Zeitung (Berlin) 16(15): 21, 1858 ≡ *Anthoceros breutelii* Gottsche, Syn. Hepat.: 583. 1846. Type: Ilha Santa Croix, near Friedenthal, *Breutel s.n.* (holotype G00115584!, PC0102910), *syn nov.*

= *Notothylloides* Asthana AK & Srivastava SC, 1991. Indian Hornworts. Type: *Notothylas levieri* St. ex Schiffn. Species Hepaticarum 5: 1021 (1917), India. W. Hengal. Kurseong, Chuttakpur forest (alt. ca. 7000-7200 ft.) Decoly & School 18906. Oct. 26. 1899 & Jan. 4.1900 (G), *syn nov.*

Diagnostic key of the genus *Notothylas* Sull.

1. Plants with sporophytes having persistent columella - 2
1. Plants with sporophytes entirely lacking a columella - 14
2. Plants prostates without long lobes, not forming rosette - *Notothylas anaporata*
2. Plants with lobes forming a rosette or flabellate - 3
3. Plants flabellate or rosette, not dichotomous lobes, with one chloroplast per cell - *Notothylas breutelii*
3. Plants forming rosettes, with dichotomous or fasciculate lobes, with one or more chloroplast per cell - 4

4. Thalli rosulate with fasciculate lobes; Pseudoelaters lacking or not well developed, when present without thickenings; inner surface of the spore with a central hollow - 5
4. Thalli rosulate with dichotomous lobes; Pseudoelaters present; inner surface of the spore with a central hollow - 7
5. Epidermal cells of capsules extremely thick-walled (7.5-10 mm), narrowly rectangular; pseudoelaters often present at releasing stage - *Notothylas depressispora*
5. Epidermal cells of capsule slightly thick-walled (2.5-4.5 mm); subquadratic to sub rectangular; pseudoelaters lacking or disintegrated at releasing stage - 6
6. Outer surface of the spore bearing central hump-like structures - *Notothylas frahmii*
6. Outer surface of the spore convex without central hump-like structures - *Notothylas irregularis*
7. Plants erect to prostrate ascending; cells of epidermal layer of the capsule wall with localized thickenings; spores 36.5-43 um with finely vermiculate sporoderm - *Notothylas himalayensis*
7. Plants ever prostrate; cells of the epidermal layer of the capsule wall with simple or stratified; spores with coarsely vermiculate or baculate sporoderm - 8
8. Spore with baculate surface - 9
8. Spore with vermiculate surface - 11
9. Thalli with dorsal ridges, occasionally lamellate; cells of the epidermal layer of the capsule wall stratified, sheet like thickenings; spores 48-66 um baculate surface with prominent denticulate flange - *Notothylas indica*
9. Thalli without dorsal ridges, lamellate or prostrate; cells of the epidermal layer of the capsule wall with simple sheet like thickenings - 10
10. Thalli profusely lamellate; spores 40, 5-50 um with baculate surface - *Notothylas galapagensis*
10. Thalli prostrate; spores 26-37 um with finally baculate surface - *Notothylas granulata*
11. Spore when mature brown with inconspicuous scar on outer surface - 12
11. Mature spores yellow with conspicuous scar on outer surface - 15
12. Sporophyte without a dehiscence line - *Notothylas temperata*
12. Sporophyte opening by a dehiscence line - 13
13. Spores 25-36 um, with depression in the center - 14
13. Spores 40-50 um, without depression in the center - *Notothylas udarii*
14. Spores light brown 27-30 um, with tuberculate surface, pseudoelater without ellipsoidal - *Notothylas. dissecta*

14. Spores 26-38 μm , with vermiculate surface, pseudoelater with ellipsoidal - *Notothylas vermiculata*
15. Sporophyte opening by a dehiscence line, pseudoelater present - 16
15. Sporophyte opening by rupture, pseudoelater absent - *Notothylas yunnanensis*
16. Spores with depression - *Notothylas pandei*
16. Spores without depression - *Notothylas orbicularis*
17. Sporogonium cleistocarpic, lines of dehiscence absent, pseudoelaters present or absent - 19
17. Sporogonium bivalved, lines of dehiscence present; pseudoelaters always present - 18
18. Spores 37.5-46.4 μm , occasionally with crescent shaped or semilunar raised structures on outer surface - *Notothylas kashyapii*
18. Spores 45-60 μm , with rare equatorial crassitudo on outer surface, exine finally vermiculate - *Notothylas javanica*
19. Thallus lamellate; involucre cylindrical with truncate opening; sporogonium emergent; spores 37.5-60 μm ; pseudoelaters absent - 20
19. Thallus smooth; involucre ellipsoid-pyriform, with apical circular-trilipped opening; sporogonium fully immersed; spores 43-55 μm with a cupulate projection on outer surface; pseudoelaters (when present) without thickenings - *Notothylas pfleidereri*
20. Spore yellow 32-45.5 μm , smooth surface, without ornamentation, without a crassitudo on outer surface, opening of the capsule by dehiscence line of two to four rows of cells; Lining layer of the capsule wall devoid of any thickening - *Notothylas nepalensis*
20. Spore brown 30- 45 μm , tuberculate surface, with some ornamentation, with crassitudo on outer surface, opening of the capsule by dehiscence line of two to three rows of cells; Lining layer of the capsule wall with transverse, spiral-annular thickening bands - 21
21. Spore 32.5-43.2 μm with united pseudoelaters, capsule usually reddish, opening of the capsule by dehiscence line of 2-3 rows of cells - *Notothylas flabelata*
21. Spore 29.5-45 μm with separate and dispersed pseudoelaters, capsule usually brown to yellowish, opening of the capsule by dehiscence line of 2 rows of cells - *Notothylas khasiana*

Descriptions and comments

Notothylas anaporata Udar & D. K. Singh, Rev. Bryol. Lichénol. 45: 202. f. 1. 1979. Type: India, Khandala, Western Ghats, November 1995, S. K. Pande WG-500 (holotype LWU).

Description and illustration - Udar & Singh (1979), Asthana & Srivastava (1991) and Singh

(2002).

Thalli prostate, dichotomously branched, usually not forming rosettes, caespitose. *Nostoc* colonies rare, subglobose. Capsules dehiscing longitudinally from apex downwards along ventral suture only, with 2(-3) cells thick, capsule wall 3-4 (-5) cell layers thick, cells of the epidermal layer reddish brown, quadrate-rectangulate. Single chloroplast with pyrenoid to the center. Spore dark brown 37.8-45.3 μm , tuberculated surface dorsal view with long protuberance, ventral view with granular surface, without depressions to the center. Pseudoelater present, columella well developed, opening of the capsule by dehiscence line of one to two rows of cells.

Notothylas anaporata usually grows on red soil, under shady and humid conditions, in association with *Cyathodium aureonitens* (Griffith) Mitt., it is a endemic plant to India in Khandala. *Notothylas anaporata* is extremely rare, known only by the type collection (Singh, 1999, 2001, 2002). Mainly characterized by the columella well developed and persistent, with oblique to spirally thickened surface cells; the spores are blackish brown, coarsely granulose with cupulate projection smooth on outer surface, and the pseudoelater with spiral thickening bands.

Notothylas breutelii (Gottsche) Gottsche, Bot. Zeitung (Berlin) 16(15): 21, 1858 $\equiv$ *Anthoceros breutelii* Gottsche, Syn. Hepat.: 583. 1846. Type: Ilha Santa Croix, near Friedenthal, *Breutel s.n.* (holotype G00115584!, PC0102910).

= *Notothylas amazonica* Spruce, Trans. & Proc. Bot. Soc. Edinburgh 15: 578. Type: Andes Peruviani, prope Tarapoto, Spruce s.n. (holotype G00115590, photo!), syn. fide Schuster 1992: 854.

= *Notothylas cubana* Steph., Sp. Hepat. 5: 1020, 1917. Type: CUBA, Aguacate, Bayamo, C. Wright s.n. (holotype G00069716!), syn. fide Schuster 1992: 854.

Description and illustration: Figure 3. B-H and Schuster (1992).

Plants flabellate with single chloroplast with pyrenoid at center. Sporophyte with quadratic to rectangular walls cells, orange, brown or pale brown. Capsule opening by a dehiscence line of two rows of cells. Spore dark brown, proximal with baculate surface, concavo, distal with apparent protuberance. Pseudoelater present, columella well developed.

Very common *Notothylas breutelii* grows on moist soil and rock at 800 - 1000 m a.s.l., always in undisturbed area. In the examined specimens it may grow associated with other bryophytes such as *Fissidens* spp. and *Targionia hypophylla* L. Distribution as tropical American species, in Brazil (Gradstein and Costa 2003) reaching as far north as Mexico and

Louisiana (Pagán, 1939; Schuster, 1992). Also reported from Hawaii (Miller, 1967) and the Philippines (Hasegawa & Tan, 1986). From the West Indies known from St. Croix, Virgin Islands (type), Puerto Rico, Cuba (also as *N. cubana* Steph.), Dominican Republic, and Guadeloupe (Frahm, 2012; Lavocat Bernard & Schäfer-Verwimp, 2011; Pagán, 1939).

Notothyas depressispora J. Haseg., Acta Phytotax. Geobot. 30: 26. 1979. Type: Thailand. Chiang Rai province, Doi Tung Mt., north of Chiang Rai, ca. 1000 m alt., middle elevation of the mountain, on soil, 24 September 1967, N. Kitagawa T-12394 (holotype KYO; isotypes G, L, NICH).

Description and illustration: Hasegawa (1979) and Chantanaorrapint (2015).

Rosulate plants with single chloroplast with just one pyrenoid in the center. Sporophyte epidermal cells very thick walls, arranged regularly, rectangular. Opening of the capsule by dehiscence line of one, rarely two rows of cells. Yellowish brown spore 30-32.5 µm, finely vermiculated surface, with bulge in the dorsal view, and vermiculated with depression in the center. Pseudoelater present, columella poorly developed, when not absent.

This species grows on soil ca. 1000 m above sea level. It is endemic to Thailand (Hasegawa, 1979). Usually identified by the epidermal cells of capsule narrow rectangular and strongly thick walled, the presence of thick walled dehiscence lines on the capsule, each inner surface of spores with a central hollow, and the outer surface with a large hump-like projection.

Notothyas dissecta Steph., Sp. Hepat. 5: 1020. 1917. Type: Central America, Majasenanga, Guatemala, *Bernoulli* 733 (holotype G00113205).

Description and illustration: Figure 10 A-F, Hässel de Menendez (1976) and Udar & Singh (1979).

Rosulate plants, with just one chloroplast with pyrenoid at the center. Capsules dehiscing longitudinally from apex downwards usually along ventral suture only with 2 cells; capsule wall 3-4 cell layers thick; cells of the epidermal layer deep brown, subquadratic to rectangular. Opening of the capsule by dehiscence line of two rows of cells, with the sinuous outer surface. Spore brown 27.5-30 µm, tuberculate surface, proximal view tuberculate with depression in the center, distal view with hump like structure. Pseudoelater present well developed columella, with helical thickening.

Notothyas dissecta usually grows on moist soil under shaded conditions. *Notothyas*

dissecta is a cosmopolitan specie, occurrence in India (Agumpe, Pune, nongthymmai) and in Central America in Guatemala. *Notothylas dissecta* are the biggest plant of the genus forming rosettes up to 3cm in diameter, the involucre longitudinally 5-8 plicate. The spores surface prominently tuberculate, the distal surface with a centrally raised area, the proximal surface with tubercles radiating from a central hollow. Pseudoelaters with prominent spiral thickening bands.

Notothylas flabellata Steph., Sp. Hepat. 5: 1020. 1917. Type: Angola, Pungo Andongo, Welwitsch, F. s.n. 1856.05 (holotype G00066854!).

= *Notothylas levieri* Schiffn. ex Steph. Sp. Hepat. 5 (Beil.): 1021. 1917. Type: INDIA. Eastern Himalaya, Kurseong, October 1898, Decoly & Schaul s.n. (holotype G-18906, isotype M). *syn. nov.*

Description and illustration: Figure 11 A-F, Hässel de Menendez (1976), Chantanaorrapint (2015) and Kobtor (2018 as *N. levieri*).

Flabellate plants, or growing as fans, the stalk is broad and long, with overlapping of the plant itself, light green, but with dark portions, marking the presence of Nostoc, and when fertile with numerous capsules, mainly on the margins of the thallus. Chloroplast 1-2 in disked shape, occasionally lobulated, with 1 (-2) region does not differentiate from the pyrenoid. Sporophyte with thickened wall cells, arranged regularly, long and rectangular. Capsule opening by dehiscence line of four rows of cells, two in each valve. Columellate.

Spore dark brown almost black 32.4-43.2 µm, tuberculate surface with slight protuberance in the distal view, tuberculated surface on both surfaces, proximal view with distinct trilete mark, without hollows at the center. Pseudoelater absent.

On moist soil or sandy rocks in shaded environments, loosely fixed to the substratum, at altitudes between 1000 and 2200 m. This species often grows associated with other bryophytes such as *Fissidens* spp. Occurrence in Angola, China, India, Nepal, Thailand (Asthana & Srivastava, 1991; Singh, 2002; Lai *et al.*, 2008; Peng & Zhu, 2014). Mainly characterized by the absence of a columella, a dark brown spores with the surface tuberculate and outer surface of the spores with 2-3 globular projections, the dehiscence line with 4-8 rows of thick walled cells.

The type of *N. flabellata* type was analyzed with sample pictures of *N. levieri*, and was identify a close proximity of the similitude with this species. *Notothylas flabelatta* and *N. levieri* were described by Steph (Species Hepaticarum vol. 5), and both species occurs in Asia. It is not the first time of are proposed a synonymization, Hässel de Menendez in a study to present

taxonomic problems with bryophytes (1976), she briefly states that these two species have the same characteristics for the spore. She analyzed the prolog and others works to verify the features of the two plants, and morphologically by the spores dark brown with exine surface tuberculate and the outer surface of the spore with a globular projection, for this the synonymization it is being done, but the molecular tools may present crypt species, like occur in others species.

Notothylas frahmii Chantanaorrapint, Cryptog. Bryol. 36(3): 254. 2015. Type: Thailand. Tak province: Umphang district, Umphang Wildlife Sanctuary, Tee Lor Su Waterfall, 12 August 2013, *Chantanaorrapint & Promma 2735* (holotype PSU, isotype BKF).

Description and Illustration: Chantanaorrapint (2015).

Fasciculate orbicular or rosettes thallus plants. Chloroplast solitary, with pyrenoid present. Involucres usually solitary, spreading horizontally or slightly ascending, conical to cylindrical, rather thick, longitudinally plicate or lamellate. Capsules cylindrical or elliptic oblong, dehiscence line with 2-3 rows of thick walled and reddish brown cells; capsule wall 2-3 (-4) cell layers thick; Columella well developed. Epidermal cells subquadratic to rectangular. Yellow spore 28-32.5 µm, finely vermiculate surface with protuberance in the dorsal view, and vermiculate with depressions on the outer surface. Pseudoelater absent.

Usually found growing with other hornworts, on disturbed soil along a walking trail ca. 300-500 m above sea level. *N. frahmii* is known only from Thailand, the type locality (Chantanaorr. 2015). *N. frahmii* is similar to *N. irregulares* Chantanaorr., but differs in the outer surface of the spore bearing a hump-like projection at its center, and is characterized by each inner spore surface with a small central hollow.

Notothylas galapagensis M. Howe, Proc. Calif. Acad. Sci., ser. 4, 21: 203. 1934. Type: Galapagos Islands, James Bay, nov.1931, *J.T. Howell 187* (holotype CAS215004).

Description and illustration: Howe (1934), only the capsule cells.

Rosulate, prostrate plants. Sporophyte with very thick wall cells. Single chloroplast. Spore yellow 40-50 µm, baculate surface without protuberance in dorsal view and with well-marked trilete mark in outer surface, slightly baculate surface. Pseudoelater present, columella well developed present, capsule opening per dehiscence line of one to two rows of cells.

Notothylas galapagensis grows associated with *Riccia* spp. The isolated plant in Galapagos differs from the species by the spores yellow brownish with tuberculate surface, the type it is NY but not was allowed to send because the sample are very delicate. *Notothylas*.

galapagensis grows in soil, but can be found as saxicolous, the specie is not know just for the type specimen, but occur just in the island.

Notothylas himalayensis Udar & D.K. Singh., J. Bryol. 11: 451. f. 1. pl. 1. 1981. Type: India, western Himalaya, Mussoorie, alt. 2122 m, *D.K. Singh 1711 R/WH* (holotype LWU).

Description and illustration: Udar & Singh (1981), Singh (2002) and Asthana & Srivastava (1991).

Rosulate plantas, prostrate, with dichotomous branches. Single chloroplast with indistinct pyrenoid. Capsule dehiscing longitudinally along one suture only, 2(-3) cells thick, capsule wall 3-5 cell layers thick; cells of the epidermal layer yellowish brown, quadrate, subquadratic towards apex. Spore brownish yellow 36.45-43.2 μm , vermiculated surface, smooth and wavy, protubery not evident in the dorsal surface, ventral view concave without depression, but with evident trilete mark. Pseudoelater present, columella present, not very developed. Opening of the capsule by dehiscence line of two to three rows of heavily pigmented cells.

Notothylas himalayensis grows on thin soil over rock surface, with the erect plants loosely fixed with substratum, under moist and shady conditions, usually associated with other hornworts like *Anthoceros* spp. and *Phaeoceros* spp. It is an endemic plant to India in western Himalaya (Mussoorie), and Rajasthan. The main features to identify is the columella well developed, persistent with spirally thickened surface cells, the spores deep brown, finely vermiculate with a conspicuous, smooth or wavy flange.

Notothylas indica Kashyap in Kashyap & Dutt, Proc. Lahore Phil. Soc. 4: 49-54. 1925. Type: Eastern India, Calcutta, Indian Botanical Garden, 26.VII.1979, *J.Lal. 3552H/POH, 3554 R/EH* (holotype LWU).

Description and illustration: Pandé (1932), Asthana & Srivastava (1991) and Singh (2002)

Prostrate plants, with dichotomous branches, forming rosettes. Chloroplast single with pyrenoid. Capsule dehiscing longitudinally along one suture only, with 2(-3) celled capsula wall, 3-5 cell layers thick; cells of the epidermal layer deep brown, quadrate-subquadratic towards apex. Columella present well developed. Spore Dark brown 48.6-64-8 μm , baculate surface with protuberance in the dorsal view and trilete mark evident in the ventral view. Pseudo elater present light brown, 1-2 cells, subglobose, subquadratic-rectangular, with incomplete spiral.

Notothylas indica is the only species that grows on rocks soil, usually in association

with mosses species. In Lucknow was found green algae profusely attached with the under surface of the plants growing. *Notothylas indica* occur in India (Mussoorie, Dehra Dun, Lucknow, Allahabd, Central India, Pachmarhi, Tikamgarh, Mumbai, Nagpur), Pakistan (Parachhinar), and Myanmar (Yangong). Mainly characterized by involucre distally lamellate, Spores with coarsely vermiculate sporoderm pattern, and conspicuous, denticulate flange.

Notothylas irregularis Chantanaorr., Acta Bot. Hung. 56(3–4): 270. 2014. Type: Thailand. Chiang Mai, Chiang Dao District, Doi Chiang Dao Wildlife Sanctuary, (19°23'35.70"N 98°53'11.26"E, 1633 m), 9August 2012, Chantanaorrapint & Inuthai 1615 (holotype PSU, isotypes BKF, EGR, G).

Description and illustration: Chantanaorrapint (2014) and Kobtor (2018).

Rosulate, fasciculate or prostrate plants, with *Nostoc* colonies irregularly dispersed. Single chloroplast with pyrenoid Sporophyte; Capsule opening per dehiscence line of one to two rows of cells, with epidermis cells with moderately thick, irregularly arranged, subquadratic to sub rectangular. Spore brownish yellow 30-35 µm, finely vermiculated surface, with protuberance in the distal view and hollows in the proximal view. Pseudo elater absent, columella present, well developed, not helical.

Notothylas irregularis Chantanaorr., was usually found growing with other hornworts, such *N. orbicularis*, on disturbed soil along trails between 1600-1900 m in rainy season. It is a specie endemic to Thailand (Chantanaorrapint, 2014). *N. irregularis* is morphologically close to *N. yunannensis*, differs by the presence of a capsule dehiscence line and a more finely vermiculate inner surface of the spores.

Notothylas javanica (Sande lac.) Gottsche, Bot. Zeitung (Berlin) 16: 20. 1858 ≡ *Blasia javanica* Sande Lac., Syn. Hepat. Jav.: 94. 1856. Type: Indonesia. Java, D.G. Holle s.n. (holotype L0061010!).

= *Notothylas vitalii* Udar & D.K. Singh, Misc. Bryol. Lichenol. 8: 173. f. 1. 1980. Type: Brazil, Mato Grosso do Sul, munic. de Miranda, Seção de Guaicurus (20°04'S, 56°46'W), in the bottom of a dried lake, ca 8km N from the main house of Fazenda Bodoquena, 11-VI-1973, D.M. Vital 2367 (holotype SP88126!, paratypes: same locality D.M. Vital 2225, 2366 (SP), *syn. nov.*

Description and illustration: Figure 4. A-E, Hasegawa (1979, 1995), Chantanaorrapint (2015), Cargill (2016) and Kobtor (2018).

Rosulate plants. Chloroplast 1-2 with pyrenoid, but sometimes not discernible. Sporophyte; Capsule opening by rupture, absent dehiscence line, with irregularly arranged epidermal cells, rectangular to quadrate, with moderately thick wall. Spore Yellow 40-45 μm , delicately vermiculated surface, with protuberance in dorsal view, pseudoelater absent, columella present, but not well developed.

Notothylas javanica usually grows on more or less disturbed soil from 100 to 1,500 m, usually associated with *Fissidens* spp. and *Targionia* spp. The main features of *N. javanica* are the irregularly ruptured of the capsule, without a dehiscence line, absence of pseudoelaters and yellowish spores with finally vermiculate surface. Occur in China, Congo, Indonesia (Java), Japan, Philippines (Luzon), Thailand (Hasegawa, 1979; Stieperaere & Matcham, 2007; Lai *et al.*, 2008; Peng & Zhu, 2014).

Notothylas kashyapii Singh *et al.*, Indian J. Forest. 23(4): 386. f. 1–13, 2000. Type: INDIA: Western Himalaya, Dehradun, D.K. Singh 401 (holotype BSD).

Description and illustration: Sahu & Asthana (2015) and Singh (2002).

Rosulate, caespitosus plants. Chloroplast, single, large, discoid, with irregular lobules and with central pyrenoid. Sporophyte dehiscing longitudinally from apex downwards. Spore Brownish yellow 37.5-46.35 μm , vermiculated surface, protuberance in the dorsal portion, ventral view with evident trilete mark, without depressions. Pseudoelater absent, columella absent,

Notothylas kashyapii do not grows associated with other bryophytes, just in pure population on ground in most, sheltered places, 418–600 m. *N. kashyapii* is a endemic plant to India in western Himalaya (Uttarakhand-Dehradun) (Sahu & Asthana, 2015). Characterized mainly by the thalli quite massive, up to 9 cell layers thick in the middle, cleistocarpic capsules, spores yellowish brown, finely vermiculate with smooth flange, pseudoelaters absent.

Notothylas khasiana Udar & Singh, J. Indian Bot. Soc. 60: 112. f. 1–29, 1981. Type: INDIA: Eastern Himalaya, Shillong. October 1975, D.K. Singh 1045 R/SH (holotype LWU).

Description and illustration: Udar & Singh (1981) and Singh (2001, 2002).

Plants very small, delicate, compact thallus, 2-3 cells thick in the middle, rare *Nostoc*. Chloroplast single with distinct pyrenoid in two zones. Sporophyte dehiscing longitudinally usually along one suture only; sutures 4-6 cells thick; 3 cell layers thick; cells of the epidermal layer deep reddish brown, quadrate - subquadrate. Spore Dark brown 29.7-45.33

µm, tuberculated surface, dorsal surface with 2 (-3) projections, forming as a half moon. Pseudoelater yellow brown – spiral; Columella absent.

Usually *N. khasiana* grows on disturbed soil under moist and partially shaded conditions. The plants grow in associated with *Phaeoceros laevis* (L.) Prosk. and are often concealed by the larger thallus lobes of the *Phaeoceros*. *N. khasiana* is an endemic plant of the India, eastern Himalaya, Meghalaya, is extremely rare; know only by the type material (Sing, 1999, 2001, 2002). It is characterized by the apex of the involucre papilose, the columella absent, spores deep brown, tuberculate surface, outer surface view with projections, the pseudoelater with strong spiral thickening bands.

Notothylas nepalensis Singh, J. Bombay Nat. Hist. Soc. 84: 649. f. 1–28 1987. Type: NEPAL: Central Himalaya, Garhigaon (S.E. of Jumla), August 1952, O. Polunin, W.R. Sykes & L.H.J. Williams 3125 (holotype LWU, isotype BM).

Description and illustration - Singh (2002),

Prostrate plants, with dichotomous branches, not forming rosettes. Chloroplast 1 (2-3) discoid, in U-shaped, without pyrenoid. Sporophyte dehiscing longitudinally from apex downwards, sutures 2-4 cells thick; opening of the capsule by dehiscence line of two to four rows of cells layers thick; cells of the epidermal layer yellowish brown, subquadratic - rectangulate. Spore light yellowish brown 32.4-45.9 µm, surface without ornamentation, smooth, clear trilete mark in proximal surface, and without projections in the distal view. Pseudoelater present, very small and extremely undeveloped, absent columella.

Notothylas nepalensis grows in association with other bryophytes such as *Anthoceros* spp., *Phaeoceros* spp. and *Fissidens* spp. in fallow fields or along the walls, in disturbed soils. It is endemic to Nepal in Central Nepal. *Notothylas nepalensis* characterized by the absence of columella, spores yellow-purplish brown exine surface obscure, devoid of flange, pseudoelaters scarce, devoid of thickening bands.

Notothylas orbicularis (Schwein.) Sull. ex A.Gray., J. Sci. Arts, ser. 2 1: 74. 1845 [1846] ≡ *Targionia orbicularis* Schwein., Spec. Fl. Amer. Sept. Crypt.: 23. 1821. Type: U.S.A., North Carolina, Forsyth, Salem, on moist earth, *Schweinitz s.n.* (holotype PH00003638!).

= *Notothylas angolensis* Steph., Cat. Afr. Pl. 2(2): 320. 1901. Type: Angola, Pungo Andongo, F. Welwitsch s.n. (holotype G00066857), syn. fide. Jones & Harrington (1983), and Schuster (1992)

= *Notothylas decurva* (Mitt.) Steph., Cat. Afr. Pl. 2(2): 320, 1901 ≡ *Anthoceros*

decurvus Mitt. Type: Angola Pungo Andongo, 12 March 1857, *F. Welwitsch* 232 (holotype NY, isotype G), syn. fide Schuster (1992).

= *Notothylas fertilis* Milde, Bot. Zeitung (Berlin) 17: 35, 1859. Type: [Czech Republic], 1856, *Lehmann s.n.* (syntype PC0104735); s.l., *C.A.J. Milde s.n.* (syntype GOET), syn. fide Schuster (1992).

= *Notothylas japonica* Horik., Sci. Rep. Tôhoku Imp. Univ., Ser. 4, Biol. 4: 425. pl. 18: f. 1–9, 1929. Type: JAPAN. Honshu. Pref. Fukushima. Prov. Rikuzen, *Y.H. Sendai 1340* (lectotype HIRO designated by Hasegawa (1979)), syn. fide Schuster (1992) and Peng & Zhu (2014).

= *Notothylas melanospora* Sull., Amer. J. Sci. Arts, ser. 2 1: 75. 1845 [1846]. Type: United States, Ohio, Franklin Co., s.col. s.n. (holotype NY00231521!), syn. fide Schuster (1992).

= *Notothylas valvata* Sull., Amer. J. Sci. Arts, ser. 2 1: 75, 1845 [1846]. Type: United States, Hab. in humidiusculis circa Columbus Ohionis, sat frequens. -Matur. AEstate-Autumno, *W.S. Sullivant s.n.* (holotype MO2146988), syn. fide Schuster (1992).

Description and Illustration: Figure 4. F-L, Hasegawa (1979), Chantanaorrapint (2015), Cargill (2016) and Kobtor (2018).

Rosulate plants prostrate, with dichotomous branches. Chloroplast 1 (-3) with pyrenoid present or absent. Sporophyte with irregularly arranged epidermal wall cells, like other species, rectangular to quadrate, with thick wall, usually ascendent because the columella present. Capsule opening per dehiscence line of two, rarely three rows of cells. Spore yellow slightly brownish, with vermiculate surface, trilete mark presente, without protuberance in distal view. Pseudoelater present, yellow to brown. Columella well developed and persistent, with irregular helicoidal thickening bands.

Species usually founded growing with some mosses, like *Targionia* spp. and *Fissidens* spp., on disturbed soil along walking trails between 60 and 2300 m a.s.l. *Notothylas orbicularis*, is a cosmopolitan species known from America, Africa, Europe and Asia (Schuster, 1992; Lai *et al.* 2008; Peng & Zhu, 2014, Stipearrier, 2007).

Notothylas orbicularis, usually is found with sporophyte, the specie has a dark thallus, growing in rosettes, and with the capsules grown dispersed on the surface, the spore is yellow and vermiculite, the pseudoelaters, columella and dehiscence line are present and it is easy to found in a cross section.

Notothylas pandei Udar & V. Chandra, Geophytology 7: 142. f. 1–11; pl. 1, f. 1–5.

1977. Type: India, Western Ghats, Jogfalls, ca 500 m, October 1962, R. Udar & V. Chandra 528 (holotype LWU).

Description and illustration - Udar & Chandra (1977), Singh (2002), Chantanaorrapint (2015) and Kobtor (2018).

Prostrate plants, with dichotomous branches, not forming rosettes. Chloroplast 1-2 usually, having two regions of pyrenoid with verrucous margin. Sporophyte with thick-walled cells, linearly organized, rectangular shape. Spore brown to black 28-32 μm , ventral view vermiculated with depressions in the center, with small granules, trilete mark not very evident. Dorsal view with globular projections. Pseudoelater present, purple to brown, undeveloped, columella well developed, not helical. Opening of the capsule by dehiscence line of two rows of cells.

Notothylas pandei grows on moist soil and rock at 800-1000 m, and differ other species normally in undisturbed area. It may grow associated with other bryophytes such as *Fissidens* and *Targionia*. Occur in India and Thailand, it is not a threatened species but like other species are usually rare to find, (Asthana & Srivastava, 1991; Singh, 2002, Chantanaorrapint, 2015).

Notothylas pfleidereri Udar & D.K. Singh., *Lindbergia* 5: 28. f. 1. 1979. Type: India, Western Ghats, Mangalore, August, 1991., *I. Pleiderer* 61217 (holotype G12823).

Description and illustration: Udar & Singh (1979).

Prostrate-ascending plants, linear, usually not forming rosettes. Single lobulated chloroplast with small region of pyrenoid. Cleistocarpous capsule with obtuse apex; capsule wall bistratose, cells of the epidermal layer pale or brownish yellow, quadrate – subquadratic. Capsule opening by dehiscence line of three to four rows of cells.

Spore Brownish yellow 43.2-52 μm , vermiculated surface, the dorsal view with protuberance, and the outer surface with evident trilete mark. Pseudoelater rare, hyaline or yellowish brown when present with hyalian bands. Columella absent,

Notothylas pfleidereri grows in close association with *Anthoceros subtilis* Steph. with the latter profusely overlapping the former. It is an endemic plant to India (South Kanara, Maharashtra) (Singh, 2001, 2002). The mainly features to *N. pfleidereri* is the sporogonia with conspicuous ‘seta’ and ovoid - ellipsoidal, cleistocarpous capsule, the capsule wall bistratose, the columella absent, finely vermiculate spores with a large projection on outer surface, and the pseudoelater very rare, usually hyaline, devoid of any thickening.

Notothylas granulata Amélio & Peralta, D.F. Type: **Brazil**. Pernambuco: Fernando de Noronha 3/8/1978, Vital, D.M. 8341 (holotype 133199). *sp. nov.*

Illustration: Figure 5. A-K.

Prostate and linear thallus, the thallus cells has one chloroplast with pirenoid. Sporophyte is erect with thick cells walls very red, *Notothylas granulata* is morphologically similar to *N. breutelii* (Gottsche) Gottsche, a widespread species of the Americas, but the spores do not have the strongly baculate surface, and the spores are tuberculate, with only a few granules in the dorsal and ventral side. It is similar to *N. pandei* Udar et V. Chandra, with the surface of the spores vermiculate to finely granulate, but it is different due to the distal surface of the spores without globular projection.

Habitat: The type specimen was found growing with mosses such as *Fissidens veracruzensis* Pursell and *F. reticulosus* (C.M.) Mitt., in wet ravines, on disturbed soil ca. 100-300 m above sea level. **Distribution:** *Notothylas granulata* is known only from the type locality; however it may also occur in other areas in northern and North-western Brazil with similar climatic condition.

Notothylas vermiculata Amélio & Peralta, D.F. Type: BRAZIL. Ilhéus, Área do CEPEC (Centro de Pesquisas do Cacau), 15/7/1991, Vital, D.M. s.n. (SP404132), *sp. nov.*

Illustration: Figure 6. A-J.

Rosulate thallus, with just one chloroplast with pirenoid. Sporophyte with thick epidermis cells, irregularly arranged, subquadratic to sub-rectangular. *Notothylas vermiculata* is morphologically close to *Notothylas irregulares* Chatanaorrapint and *Notothylas yunannensis* T. Peng & R.L.Zhu. these species has in common with *N. vermiculata* the vermiculate spores with a small hollow on each subunit of inner surface, and sub quadrate to rectangular and the irregularly arranged epidermal capsule cells. *Notothylas vermiculata* differs from *N. irregularis* by an ornamentation vermiculate evident inner surface of the spores, and differs from *N. yunannensis* by the presence of a dehiscence line of the 2-3(-4) cells and pseudoelaters.

Notothylas vermiculata grows in disturbed soil ca. 300-500 m above sea level, over a cashew tree. *Notothylas vermiculata* is known only from the type locality; however it may also occur in other areas in northern and North-western Brazil with similar climatic condition.

Notothylas temperata J. Haseg., Acta Phytotax. Geobot. 30: 20. f. 2-3. 1979. Type: Japan,

Honshu, Pref. Kyoto: Kyoto, Sakyo-ku, Iwakura, *Hasegawa 6589* (holotype KYO; isotypes G00115588!; L; NICH).

Description and illustration: Figure 12 A-G and Hasegawa (1979).

Rosulate plants. Chloroplast solitary with pyrenoid. Sporophyte have thick epidermal cells, regularly arranged, rectangular to sub-rectangular. Capsule open by rupture, absence of dehiscence line. Spore dark brown to black (-35.5) 37.5-42.5 μm , vermiculated surface. Pseudoelater present pale yellow. Columella present, reaching more than the middle of the length of the capsule, however, sometimes indistinct.

Notothylas temperata is common in gardens and farms, and most common in rice fields, the interesting is than *N. temperata* only occur in the Pacific Ocean side (Hasegawa, 1979). Capsule open by a rupture, absence dehiscence line, with a distinct columella, spores brown with slightly vermiculate surface. *Notothylas temperata* is endemic to Japan, occur in Honshu, Shikoku, Kyushu (Hasegawa, 1979)

Notothylas udarii D.K. Singh & Semwal, Phytotaxonomy 1: 35. pl. 1–2. 2001. Type: India: Western Himalaya, Dehradun, *D.K. Singh 347* (holotype BSD).

Description and illustration - Singh & Semwal (2001) and Singh (2002).

Rosulate plants, narrow thallus. Chloroplast single with central pyrenoid. Sporophyte with thick epidermis cells, regularly arranged, subquadratic to sub-rectangular. Capsule opening per line of dehiscence of two to three rows of heavily pigmented cells. Spore dark brown 40.69-50.08 μm , vermiculated surface, dorsal view with protuberance, and evident trilete mark on outer surface. Pseudo elater present, with thick spiral bands, columella well developed.

Notothylas udarii grows on moist ground, walls as well as on flowerpots in the garden, in sheltered places, in close association with other bryophytes. *Notothylas udarii* is endemic to India in western himalaya Dehradun.

Notothylas yunnanensis T. Peng & R.L. Zhu, Phytotaxa 156: 157. 2014. Type: China. Yunnan: Mengla Co., Menglun to Mengbang, 636 km, on moist soil by road (27°49.833'N, 101°20.303'E, 1155 m), 15 July 2012, t. peng et al. 20120715-7 (holotype HSNU).

Description and illustration: Peng & Zhu (2014), Chatanaorrapint (2015) and Rattanamanee and Chantanaorrapint (2015).

Fasciculated rosulate plants. Chloroplast solitary with pyrenoids. Sporophyte with

moderately thick epidermis cells, irregularly arranged, subquadratic to sub-rectangular. Opening of the capsule by rupture, absence of dehiscence line. Spore light yellow 28-36 µm, vermiculated surface without protuberance in the dorsal view, concave with well developed trilete mark in outer surface. Pseudoelater absent, columela well developed, not helical.

Usually *N. yunannensis* grows on disturbed soil at 600-1200 m and is usually associated with other bryophytes such as *N. javanica*, *Phaeoceros carolinianus* and *Fissidens* spp. Occur in China and Thailand (Tao & Zhu, 2014; Chatanaorrapint, 2015; Rattanamanee, S. & Chantanaorrapint, S. 2015).

Excluded names from *Notothylas* Sull

Notothylas chaudhurii Nirula was described in 1945 from Nagpur, but this species publication is invalidly, as shown by Singh (2002).

Notothylas verdoornii Khanna described in 1933 from Nianmar, although the work was published without a type specimen in the prologue. For this reason, the absence of the type specimen, this species publication is invalid, and excluded to the genus.

Notothylas paroicus Schiffn. was a shared error. In the original publication attributed to this species (Denkschr. Kaiserl. Akad. Wiss., Wien Math.-Naturwiss. Kl. 67: 192. 1898), *Notoscyphus paroicus*. *Notoscyphus* is a liverwort, not a hornwort.

Synonymized names from *Notothylas* Sull

Paraphymatoceros hallii (Austin) Hässel, Phytologia 88: 209. 2006 ≡ *Phaeoceros hallii* (Austin) Prosk., Bull. Torrey Bot. Club 78: 347. 1951 ≡ *Anthoceros hallii* Austin, Bull. Torrey Bot. Club 6: 26. 1875. Non *Notothylas hallii* Austin ms., Bull. Torrey Bot. Club 6: 27. 1875. Original specimen: NY 00226641!, nom inval. Lectotype (designated by M. Howe, Bull. Torrey Bot. Club 25: 11. 1898): USA, Oregon [Marion County], 26, Springy Places, Silverton, Hall [26] (MANCH -EM74235/21217!; isoelectotypes MANCH (2!) EM74234/21216, EM74241/21224; Paratype: USA, Oregon [Marion County], Dripping rocks, Salem, Hall [35] (MANCH (2) EM74232/ 21214, EM74237/21219).

≡ *Anthoceros sulcatus* Austin, Bull. Torrey Bot. Club 6: 27. 1875. Type: USA, Oregon [Marion County], 25, moist earth, Salem, Oregon, Hall [25] (Lectotype MANCH-EM74240/21223 designated here; isoelectotypes MANCH (4!) EM74233/21215, EM74236/21218, EM154642/ 21221, EM74239/21222).

Austin (1975) note that this species is a link between *Anthoceros* and *Notothylas*, and describe a new name *Anthoceros sulcatus* Austin. Hassel makes a new combination with the new genus *Paraphymatoceros* Hässel in the same study the author makes a new description (*Paraphymatoceros diadematus* Hässel), and other combination (*Phymatoceros minutus* (Mitt.) Hässel).

Phaeoceros minutus (Mitt.) S.W.Arnell, Hepat. South Africa: 403, 1963 $\equiv$ *Phymatoceros minutus* (Mitt.) Hässel, Phytologia 88(2): 2006 $\equiv$ *Notothylas minuta* (Mitt.) Steph., Spec. Hep. V:1021, 1916 $\equiv$ *Anthoceros minutus* Mitt., J. Linn. Soc., Bot. 16 (91): 195, 1877. Type: Base of Table Mts., Cape of Good Hope, A.E. Eaton s.n. (holotype NY231447!, paratype NY231448!, 231449!).

Actually, *Phaeoceros* are the genus to this specie, after molecular studies. Doubts around this species because the sporophyte is small, and the genus *Notothylas* has the smaller sporophytes to hornworts.

Considerations

This study brought the whole survey of the genus *Notothylas* Sull. With a brief resolution about the two subgenera, through molecular studies and cladistic analysis. We have also brought the taxonomic analysis, with all the species synonymized and their respective specimen's types. This study accompanies two new species to Brazil

To type specimens of *Notothylas*, unfortunately, we can not enjoy all the specimens, because of the state of conservation in some samples are not good. The *Notothylas* samples are very sensitive plants after dry, and in a enveloped with remains of substrate it is very detrimental to morphological conservation, mainly to the gametophyte, as is the case of *N. orbicularis*, and the others samples from NY. In order to avoid further wear and tear of this material of great importance, the vegetable material were cleaned and isolated, to avoid further contact. However is very important comment than samples types as *N. flabelata*, and *N. temperata*, do not have substrate and the gametophyte it is very conservative, have sporophytes which is an indispensable structure in the identification. Unfutanable not was possible analyse all the types materials and others samples, due to measures established by the country and the institutions.

The *Notothylas*, are not a genus with risk status of the conservation in Brazil, however some species has the potential to considered extinct, because the only known sample is the type specimen, as *N. frahmii*. The *Notothylas* grows in open areas, such as little disturbed soil, above 100 m above sea level.

After numerous studies about molecular methods used in hornworts, the genus *Notothylas* present difficulties. To news answers about to subgenera and others sub generic divisions, we suggest start with news regions to find mutations more expressive. We suggest the use of the new buffer, usual to news primers (trnS and rbcS). The project about the circumscription, where the genus can be fully recognized in molecular way is still in progress, however this study we have been able to determine that the genus does not divide due to the morphological characteristics, and that it reinforces as a monophyletic genus.

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5. Illustrations

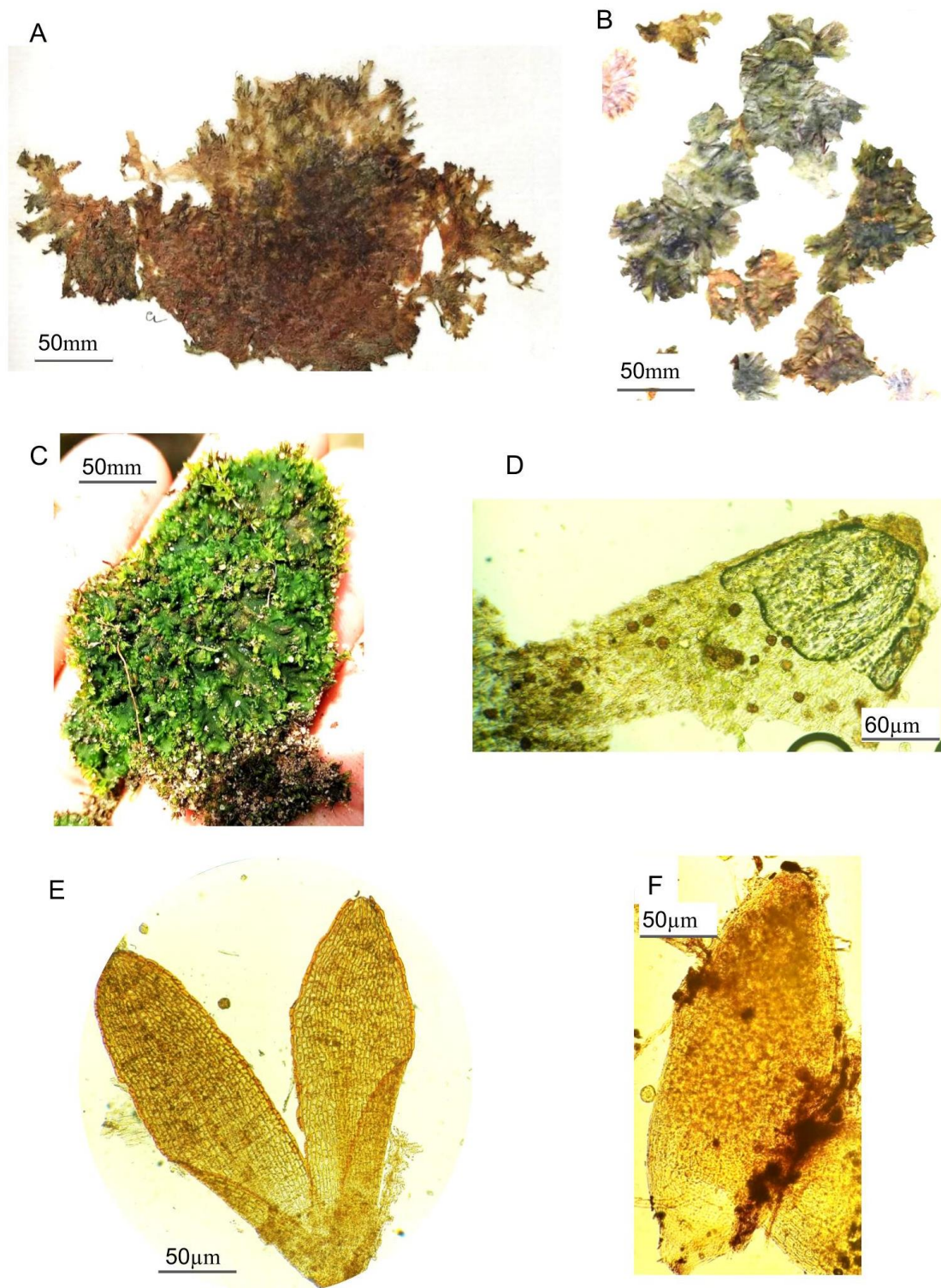


Figura 1. A-C. Aspectos dos talos. A. Talo flabelado desidratado em exsicata de *Notothylas flabellata*. B. Talo flabelado desidratado em exsicata de *Notothylas breutelii*. C. Talo rosulado em habitat natural de *Notothylas orbicularis*. D. Anterídio: Invólucro: 5- involucro com alguns esporos provenientes da cápsula. E-F. Aspectos das cápsulas. E. Cápsula valvada, com linha de deiscência. F. Cápsula, não valvada, sem linha de deiscência.

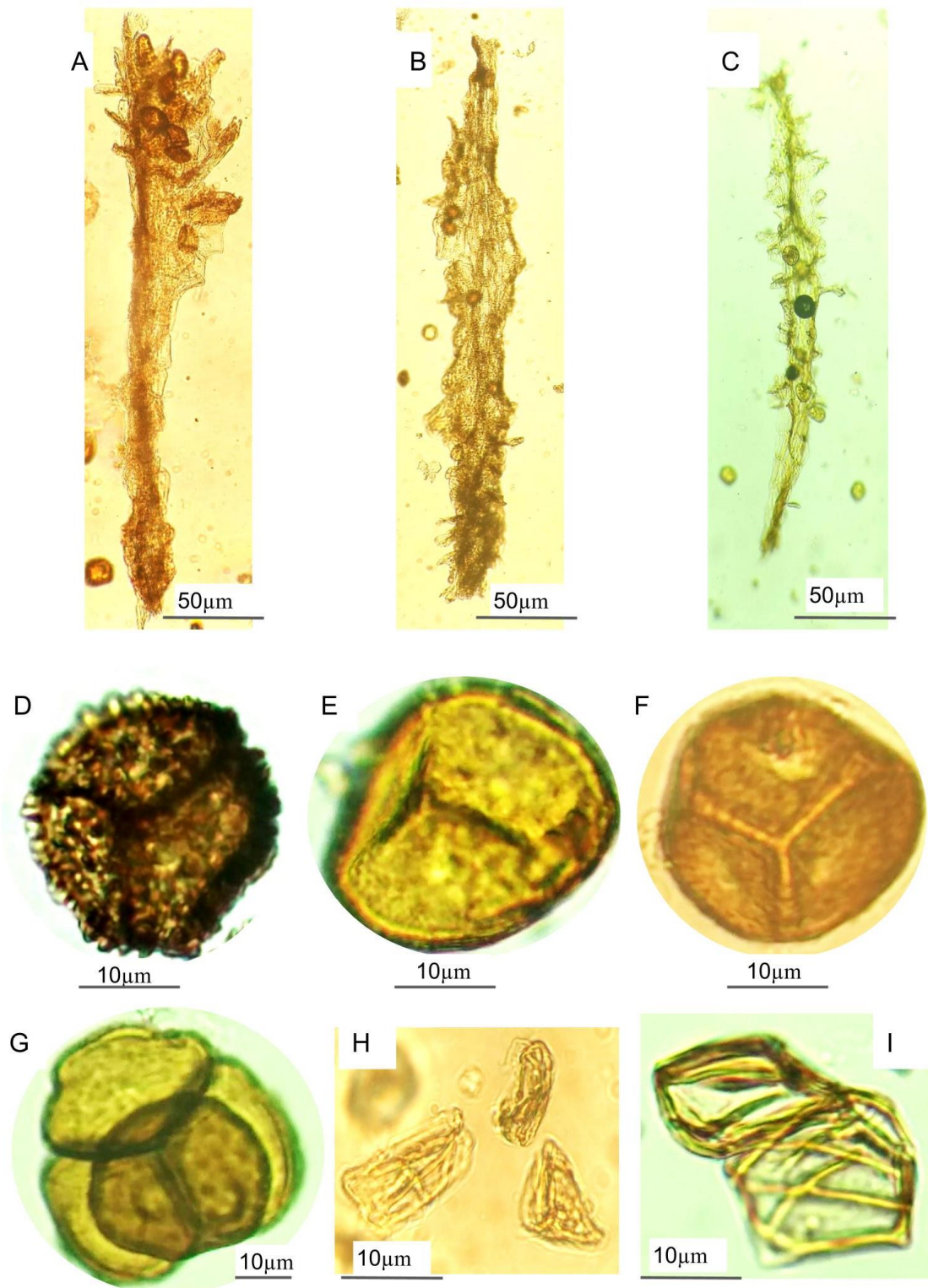


Figura 2. A-C. Aspectos das columelas. A. *Notothylas breutelii*. B. *Notothylas temperata*. C. *Notothylas orbicularis*. D-G. Aspectos dos esporos. D. Baculado. E. Vermiculado. F. Tuberculado. G. Esporos ainda em tétrede. H-I. Aspectos dos pseudoelatórios. H. Pouco desenvolvido. I. Maduro.

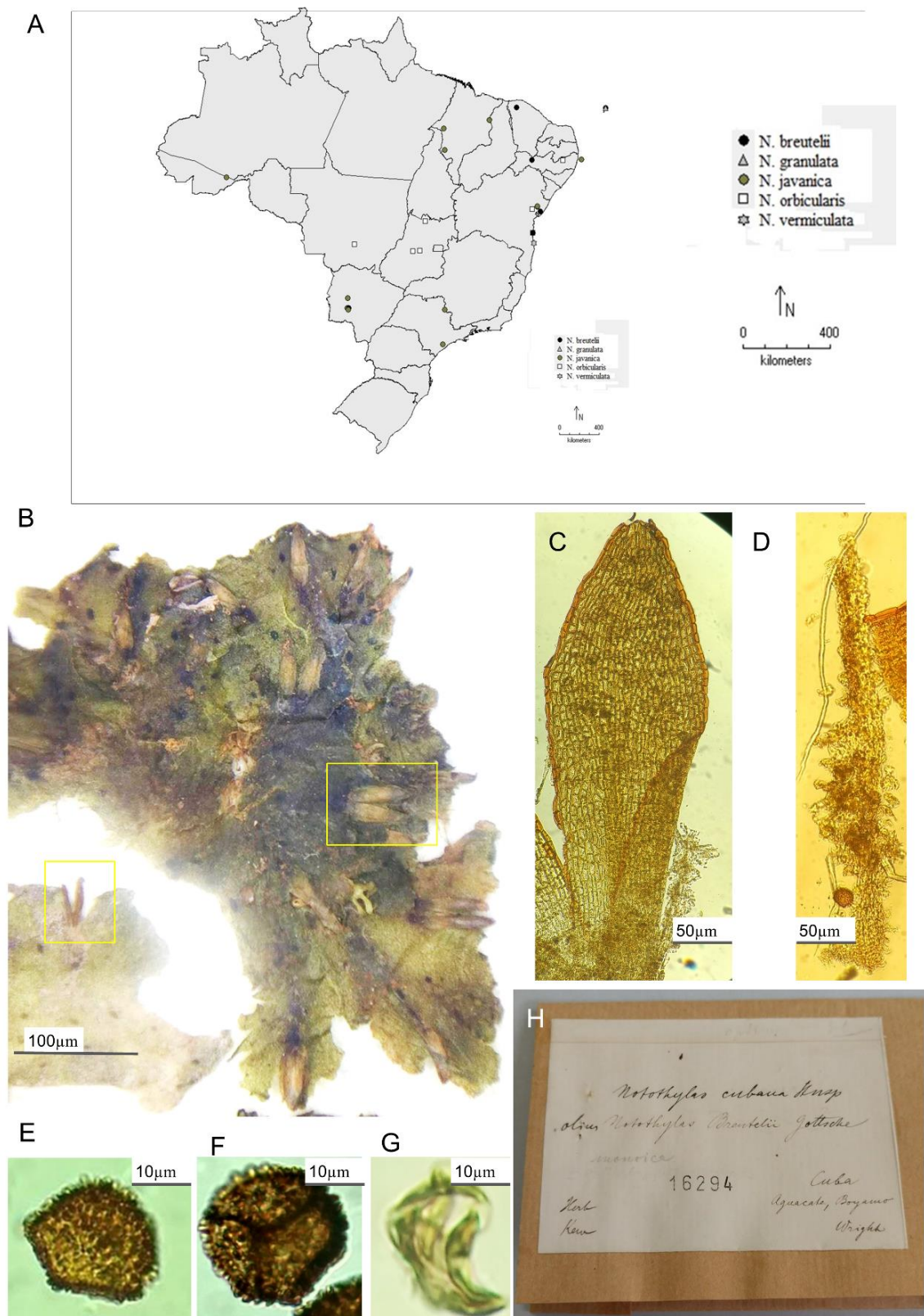


Figure 3. A. Distribution range of *Notothylas* in Brazil. B-H. *Notothylas breutelii* (Gottsche) Gottsche. B. Thallus with sporophytes. C. Capsule margin. D. Collumela. E. Spore dorsal view. F. Spore proximal view. G. Pseudoelater. H. Type of *Notothylas cubana*

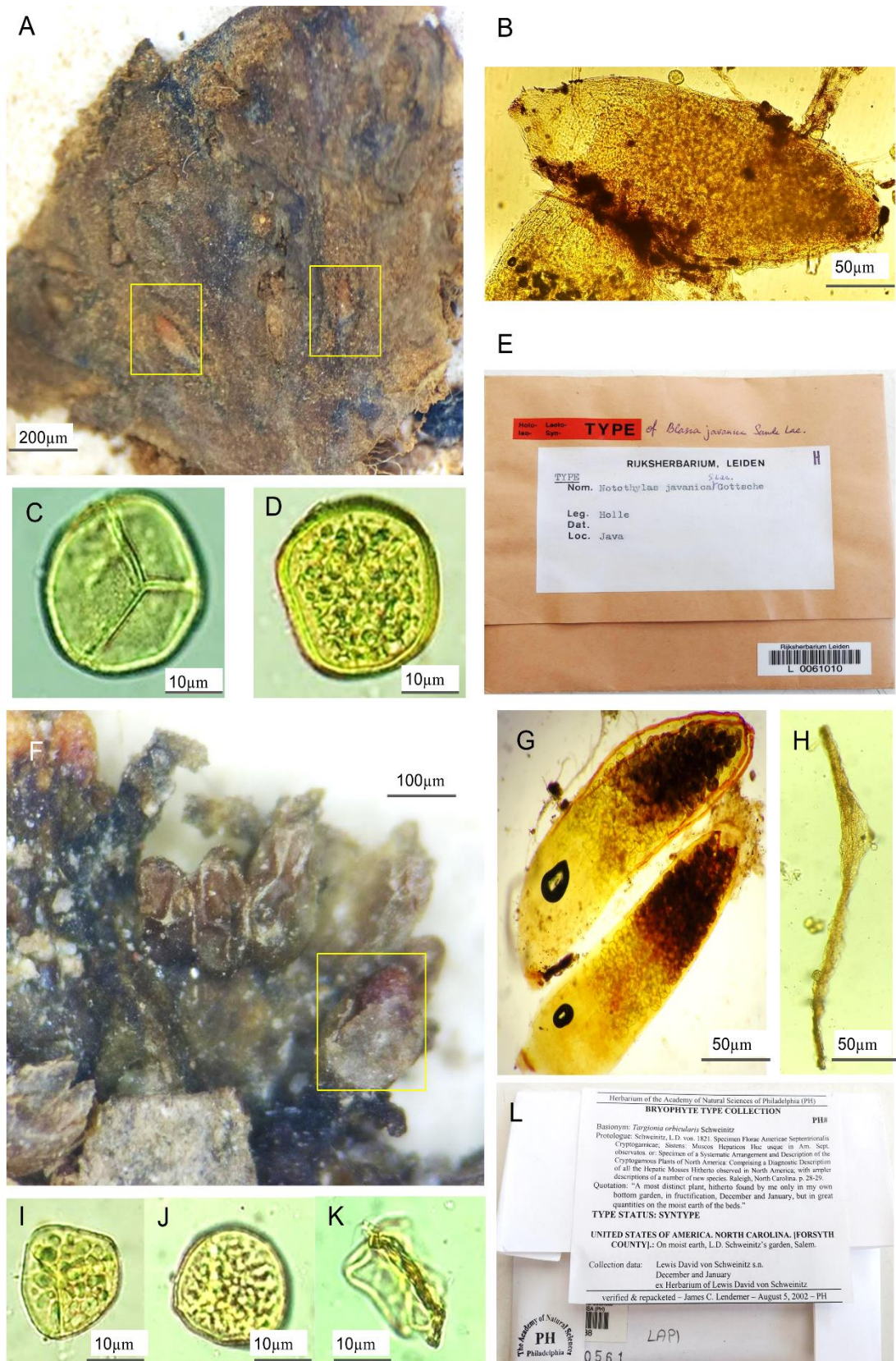


Figure 4. A-E. *Notothylas javanica* (Sande Lac.) Gottsche. A. Thallus with sporophyte. B. Capsule with spores. C. Spore proximal view. D. Spore dorsal view. E. Type specimen. F-L. *Notothylas orbicularis* (Schwein.) Sull. F. Thallus with sporophyte. G. Capsule with spores. H. Collumela. I. Spore dorsal view. J. Spore proximal view. K. Pseudoelater. L. Type specimen.

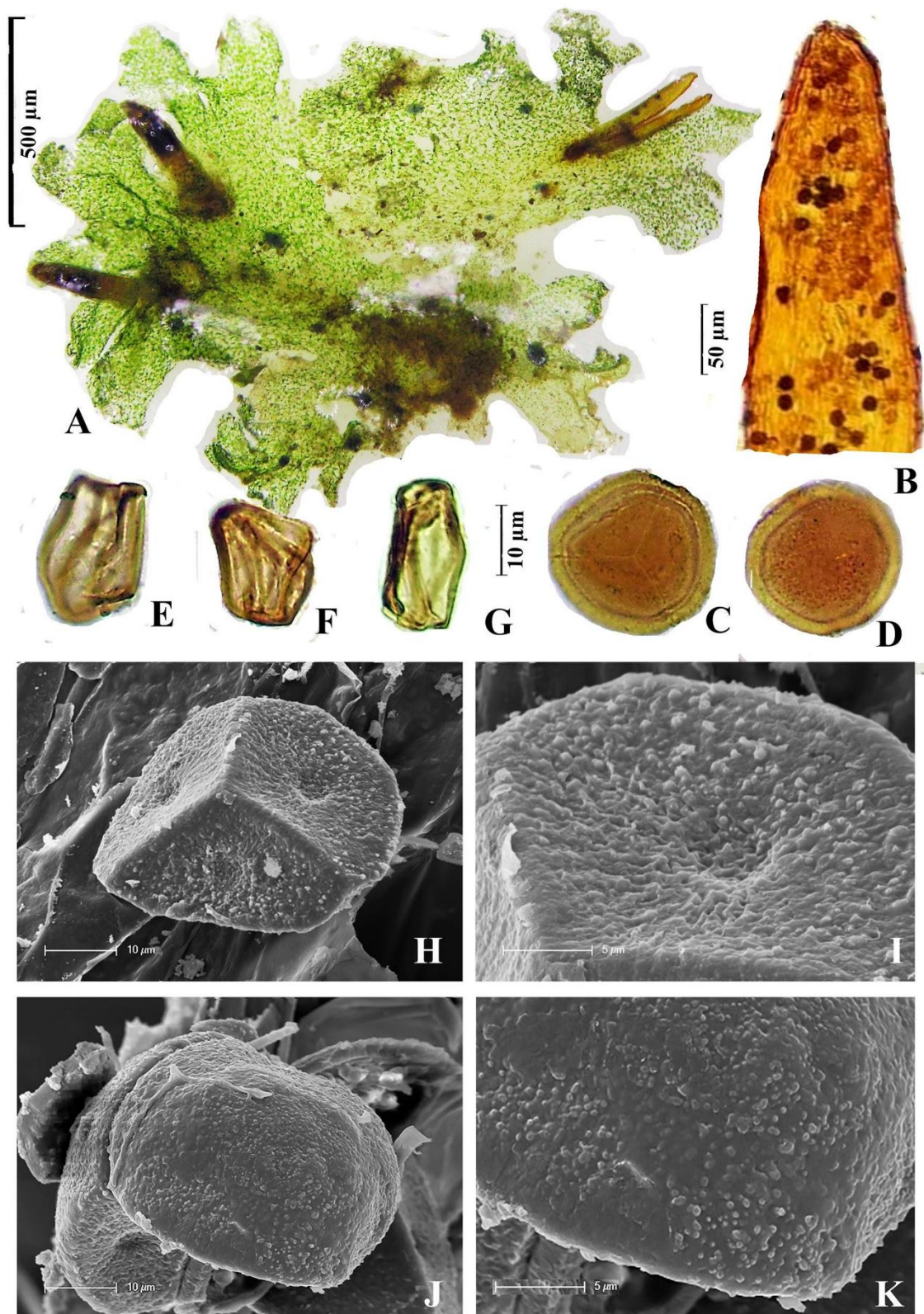


Figure 5. *Notothylas granulata* Amélio & Peralta. A. Rehydrated plant. B. Distal cells of the capsule. C. Light microscopy of the proximal spore surface. D. Light microscopy of the distal spore surface. E-G. Pseudoelaters. H. SEM of the proximal spore surface. I. Detail of the ornamentation of the hollow on the proximal face. J. SEM view of the distal face of the spore. K. Detail of the ornamentation on the distal surface.

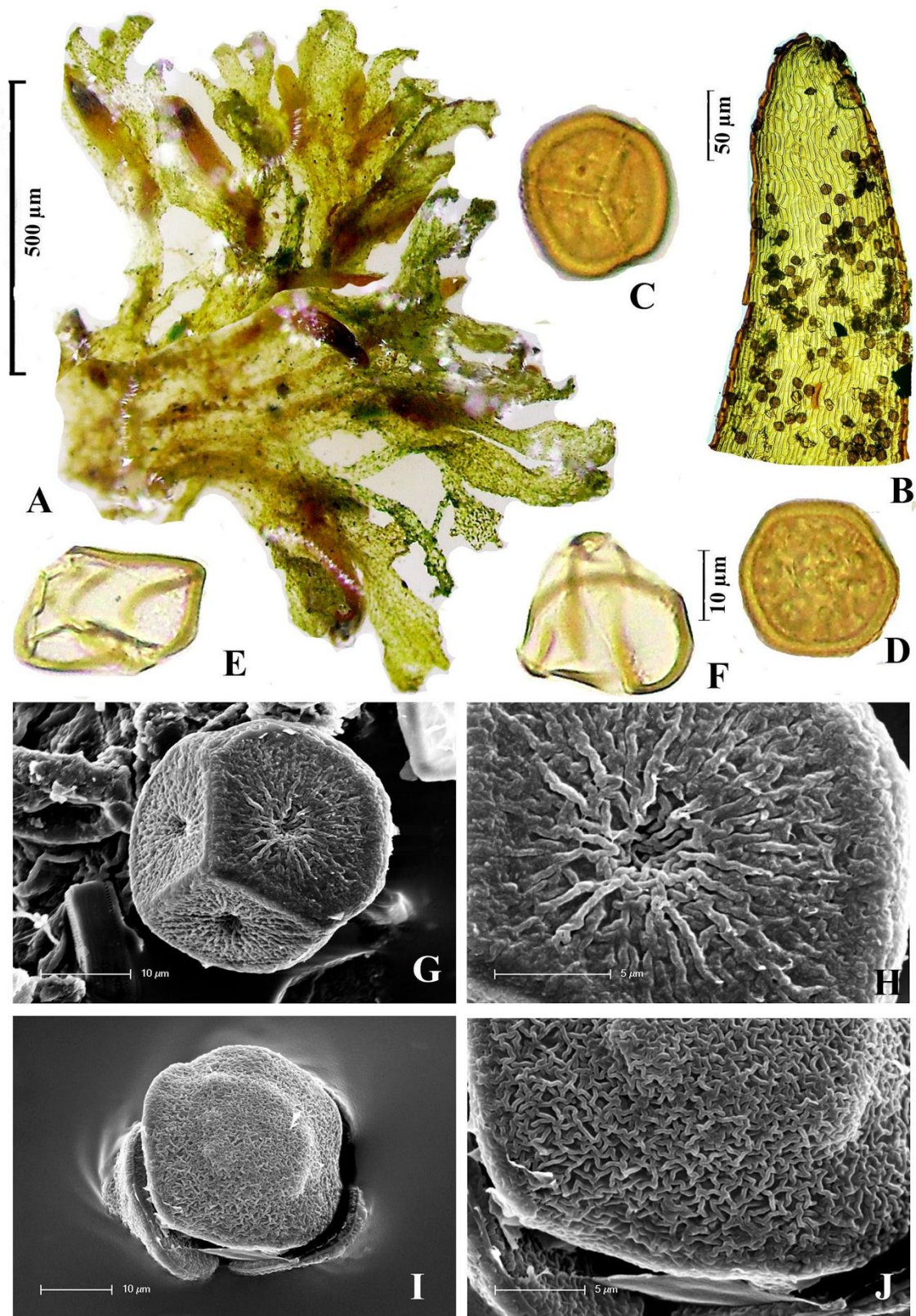


Figure 6. *Notothylas vermiculata* Amélio & Peralta. A. Rehydrated plant. B. Distal cells of the capsule. C. Light microscopy of the proximal spore surface. D. Light microscopy of the distal spore surface. E-F. Pseudoelaters. G. SEM of the proximal spore surface. H. Detail of the ornamentation of the hollow on the proximal face. I. SEM view of the distal face of the spore. J. Detail of the ornamentation on the distal surface.

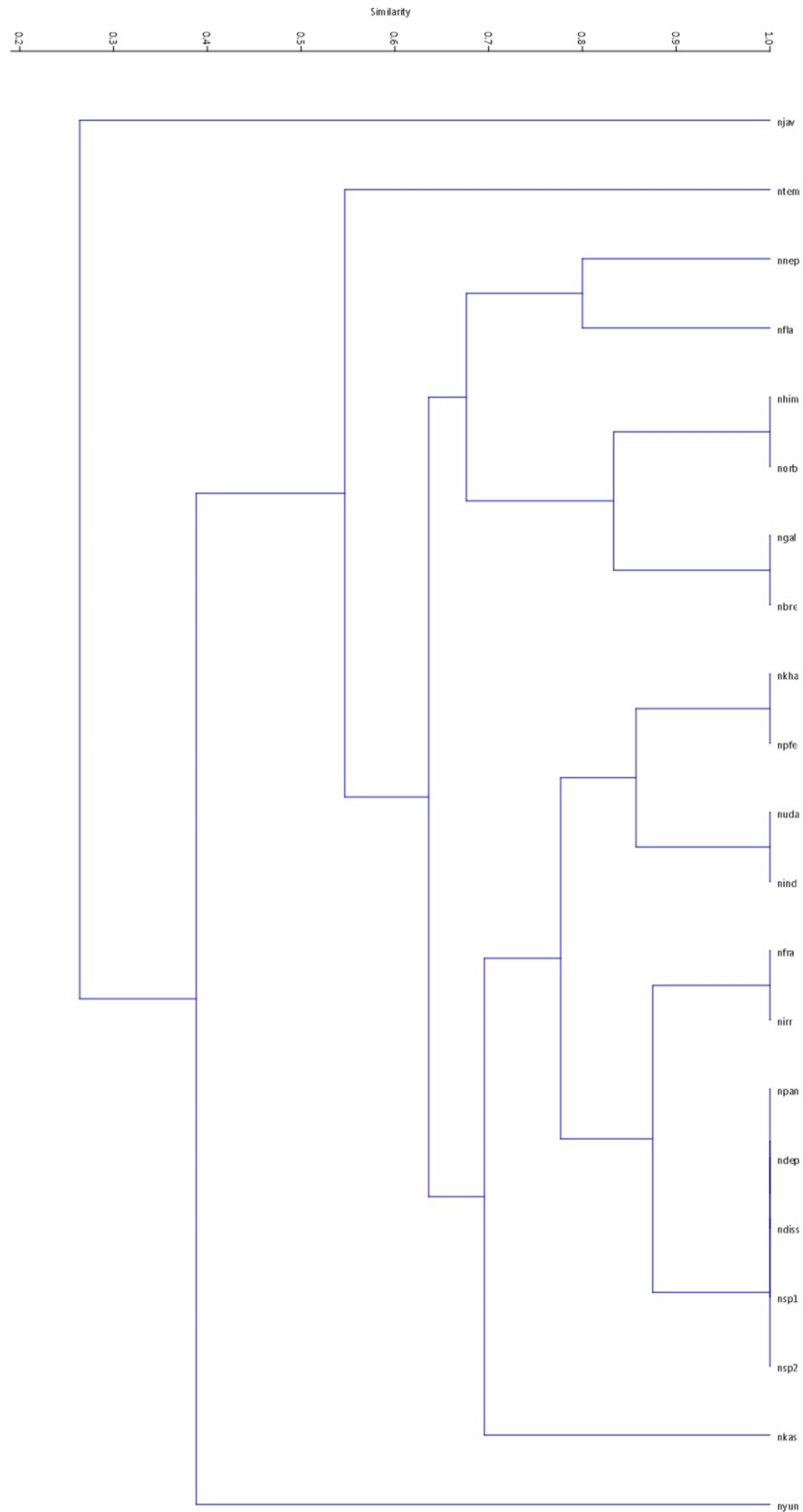
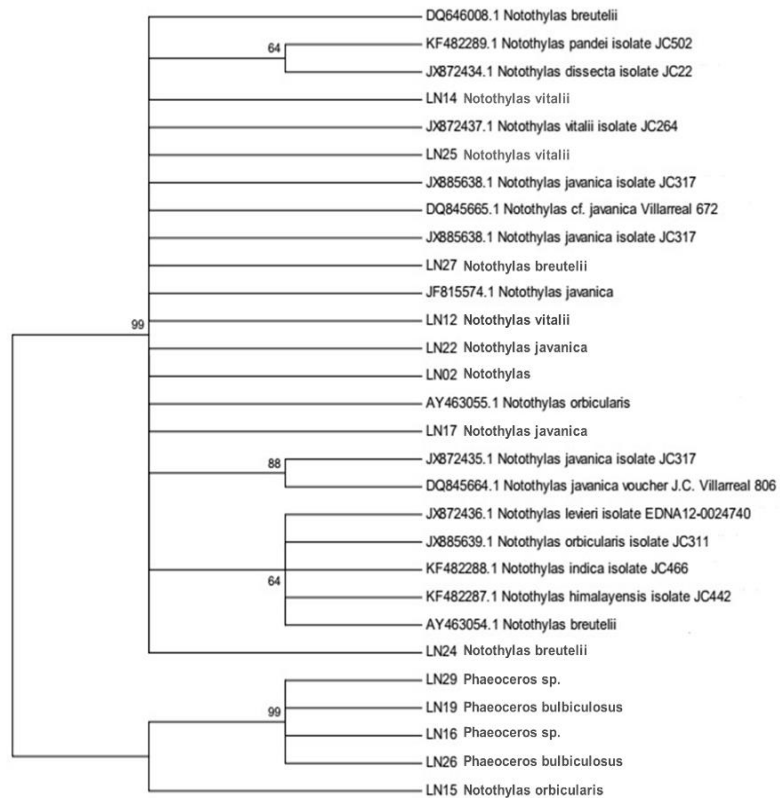


Figure 7. Cluster analysis of all 21 recognized species concerning about the morphological structures, abbreviation of species and data on based on ANEX III.

A



B

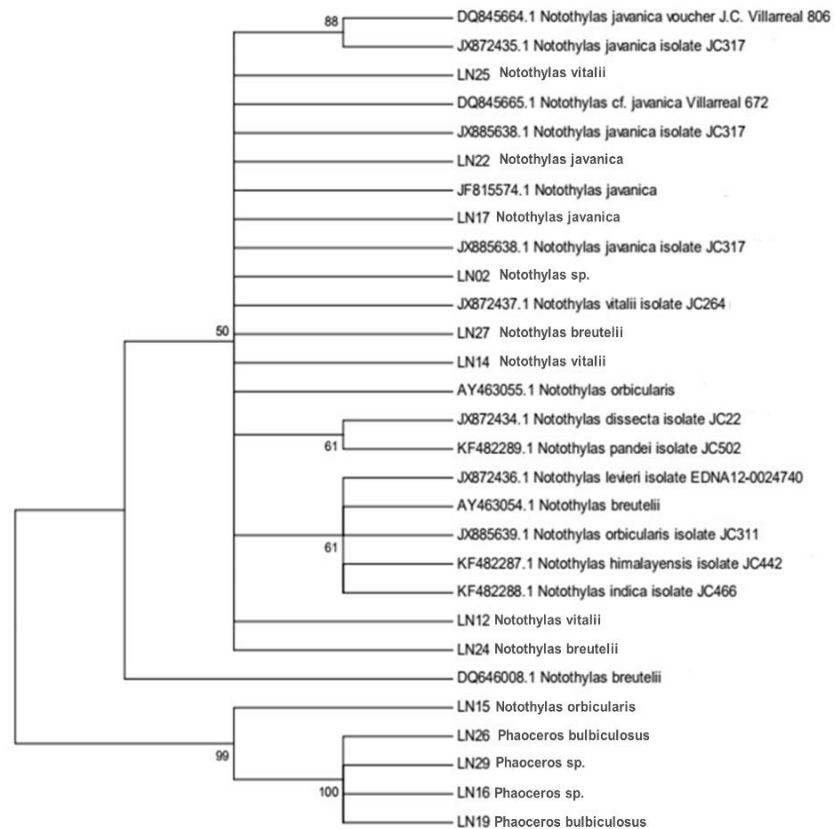


Figure 8. A. Cladogram based on a maximum parsimony analysis of *Notothylas* based on *rbcL*. B. Cladogram based on a maximum likelihood analysis of *Notothylas* based on *rbcL*.

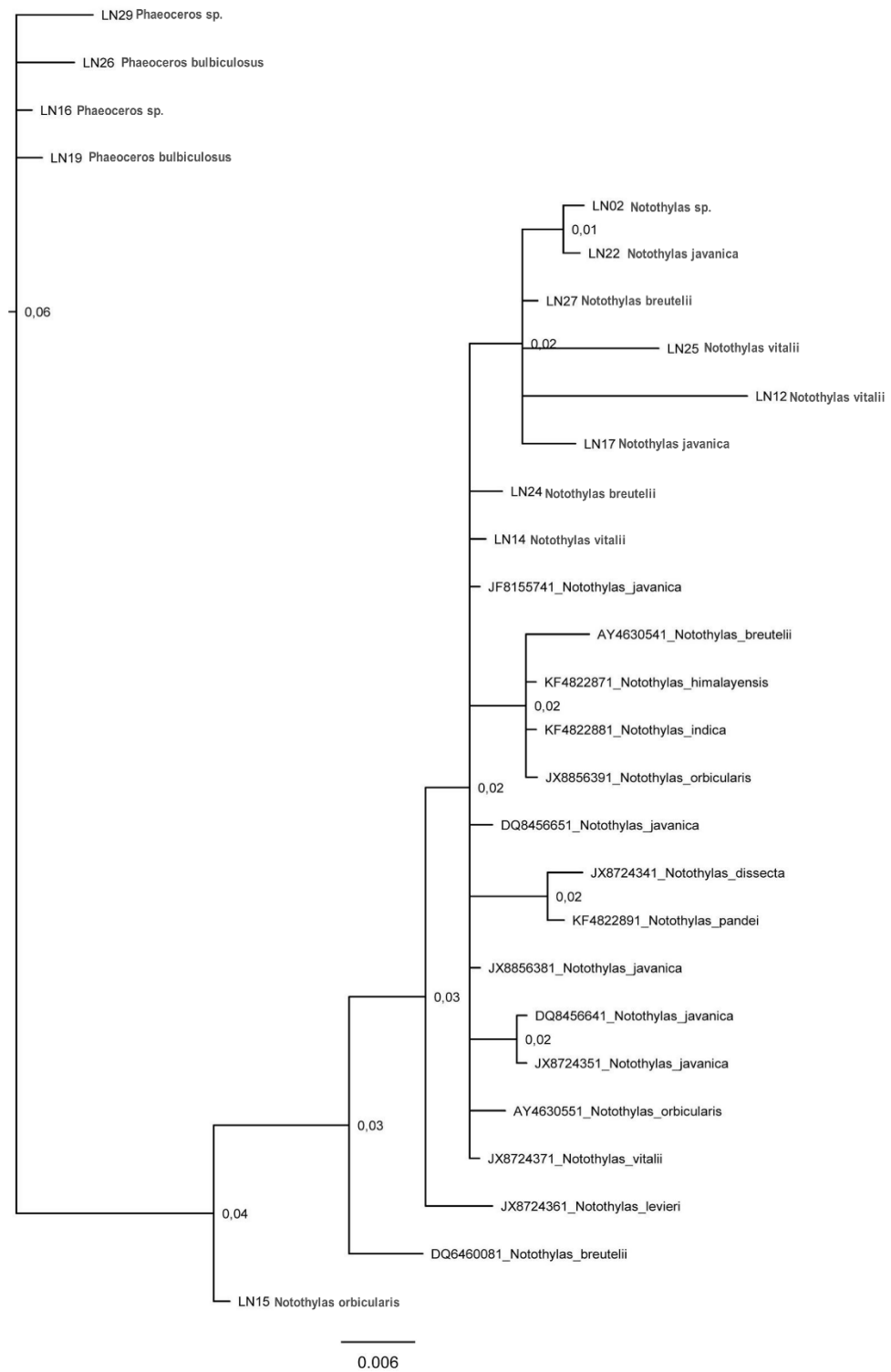


Figure 9. Filogram based on a maximum Bayesian analysis of *Notothylas* based on *rbcL*.

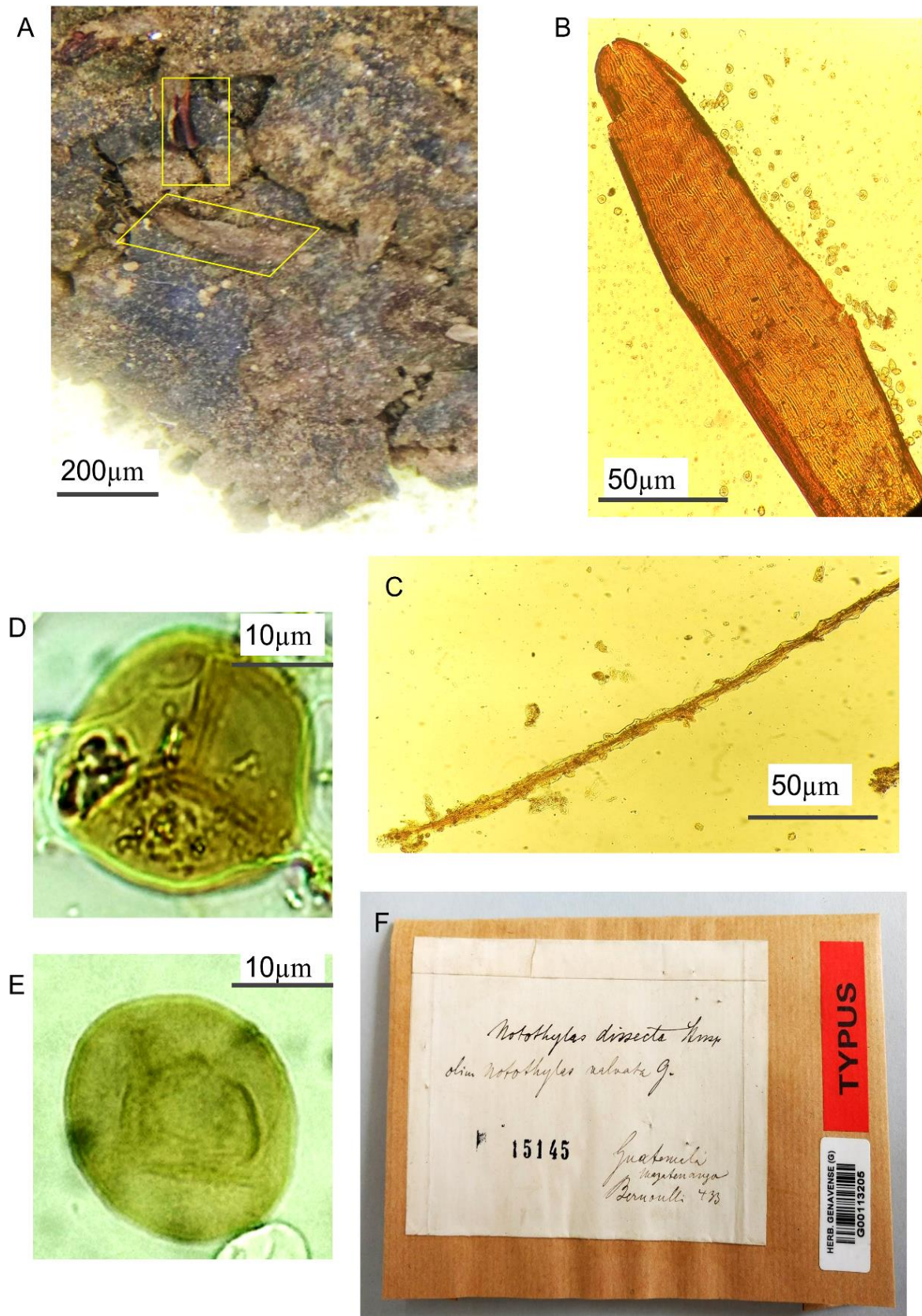


Figura 10. A-F. *Notothylas dissecta* Steph. A. Thallu with sporofites. B. Capsule margin. C. Collumela. D. Spore proximal view. E. Spore dorsal view. F. Type of *Notothylas dissecta*..

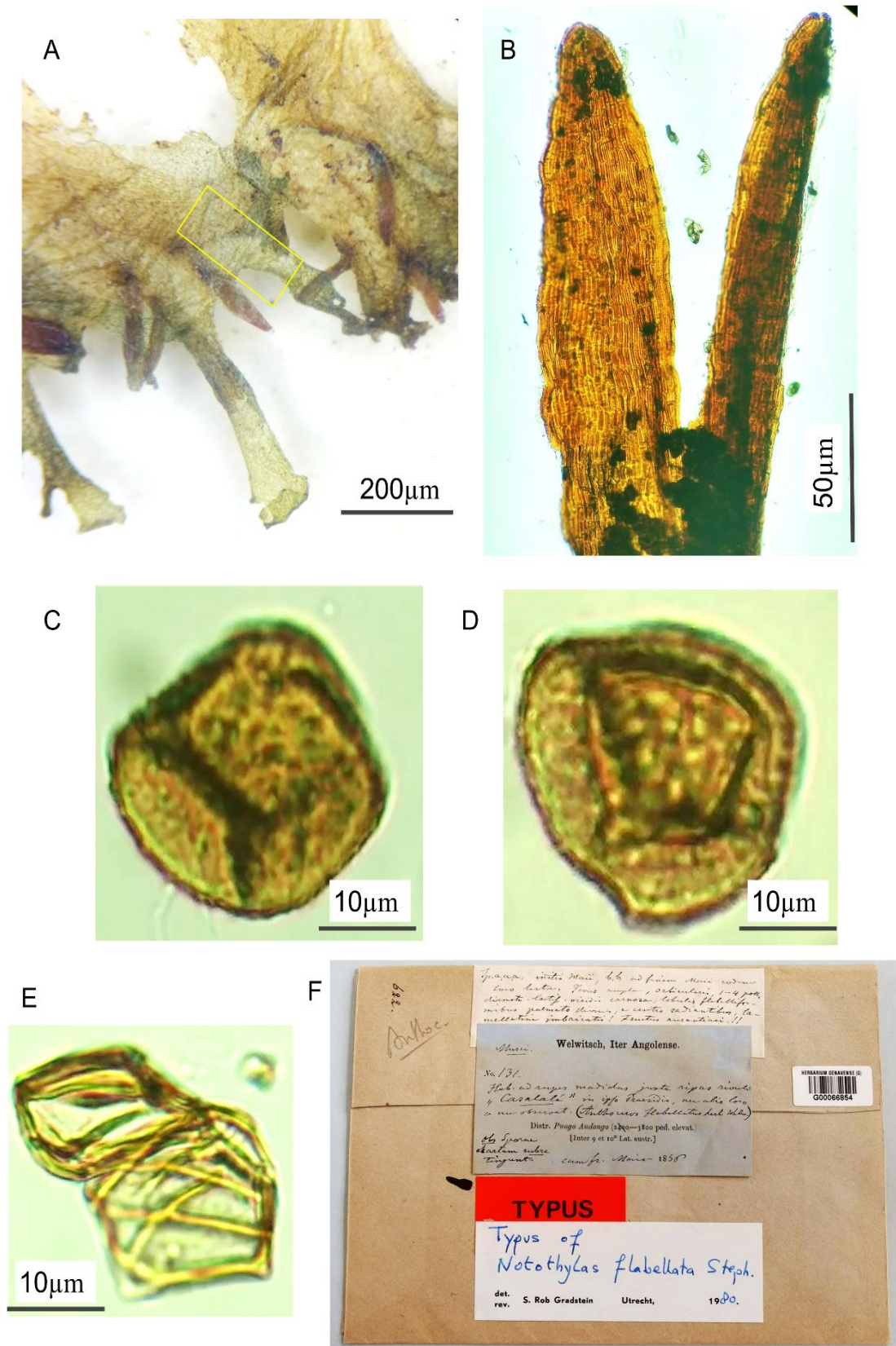


Figure 11. A-F. *Notothylas flabellata* Steph. A. Thallus with sporophytes. B. Capsule with spores. C. Spore proximal view. D. Spore dorsal view. E. Pseudoclater. F. Type of *Notothylas flabellata*.

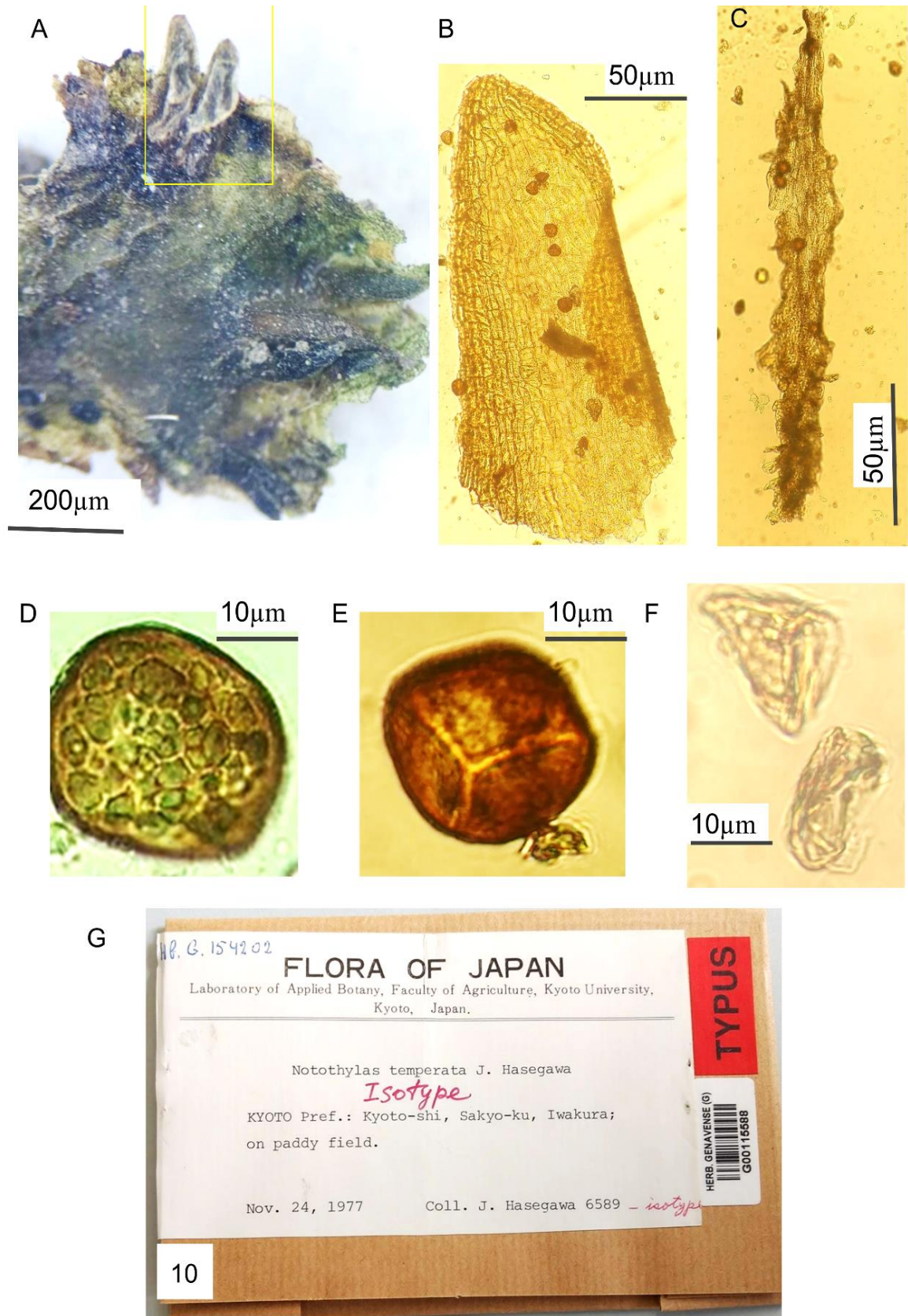


Figure 12. A-G. *Notothylas temperata* J. Haseg. A. Thallus with sporophytes. B. Capsule. C. Collumela. D. Spore dorsal view. E. Spore proximal view. F. Pseudoelater. G. Type of the *Notothylas temperata*.

6. ANEXOS

6.1. ANEXO I. Cronologia do surgimento e descrição das espécies dentro do gênero *Notothylas*

Ano	Autor/Publicação	Comentários
1845	Sullivant. Amer. J. Sci. Arts, ser. 2 1: 74	Transferência e descrição do gênero. <i>Notothylas</i> com três novas spp. e uma variedade; <i>N. orbicularis</i> (Schwein.) Sull., <i>N. melanospora</i> Sull, <i>N. valvata</i> Sull. <i>N. orbicularis</i> var. <i>orbicularis</i> . O primeiro registro foi como <i>Carpobolus orbicularis</i> em 1821, e <i>Targionia orbicularis</i> foi adotado em 1822.
1858	Gottsche. Bot. Zeitung (Berlin) 16(15): 21	Transferência de <i>Anthoceros breutelii</i> para <i>N. breutelii</i> (Gottsche) Gottsche e <i>Blasia javanica</i> para <i>N. javanica</i> ;
1859	<i>Notothylas fertilis</i> (Milde) Milde. Bot. Zeitung (Berlin) 17: 35	
1875	Austin. Bulletin of the Torrey Botanical Club 6: 27. 1875.	Descrição de <i>N. hallii</i> Austin para os E.U.A.
1885	Spruce. Trans. & Proc. Bot. Soc. Edinburgh 15: 578	Descrição da primeira sp. brasileira <i>N. amazonica</i> Spruce
1898	<i>Notothylas paroicus</i> Schiffn. Denkschr. Kaiserl. Akad. Wiss., Wien Math. Naturwiss. Kl. 67: 192	<i>Notocyphus paroicos</i> é o verdadeiro nome dessa espécie, uma hepática folhosa. <i>N. paroicus</i> nunca existiu, foi um erro que resistiu aos anos.
1901	Stephani. Catalogue of the African Plants collected by Dr. F. Welwitsch in 1853-61 2(2): 320. 1901.	Descrição das primeiras espécies africana para o gênero: <i>N. angolensis</i> Steph, <i>N. decurva</i> (Mitt.) Steph., <i>N. flabellata</i> Steph.
1917	Stephani. Species Hepaticarum 5: 1021. 1916.	Stephani reúne em seu trabalho mais famoso quatro novas descrições: <i>N. minuta</i> (Mitt.) Steph., <i>N. cubana</i> Steph., <i>N. dissecta</i> Steph. <i>N. levieri</i> Schiffn. ex Steph.
1925	Proceedings of the Lahore Philosophical Society 4: 49. 1925.	Kashyap inicia uma sequência de descrições do gênero para a Ásia, na Índia, no Paquistão e em Myanmar; <i>N. indica</i> Kashyap.
1929	Horikawa. Science Reports of the Tôhoku Imperial University, Fourth Series, Biology 4: 425. pl. 18: f. 1-9. 1929.	Horikawa descreve a primeira espécie do gênero para o Japão, <i>N. japonica</i> Horik.
1933	Khanna. Revue Bryologique et Lichénologique 6: 118. 1933.	<i>N. verdoornii</i> Khanna foi descrita como endêmica de Myanmar, porém, esta publicação nunca esteve nos padrões

- estabelecidos pelo código de nomenclatura botânico. A ausência do espécime tipo, de acordo com o código de nomenclatura botânica, anula esta espécie.
- 1934 Howe. Proceedings of the California Academy of Sciences, Series 4, 21: 203. 1934. *Notothylas galapagensis* M. Howe. caracterizada por ser endêmica das Ilhas Galápagos, esta espécie isolada se diferencia das demais.
- 1945 Nirula. Proceedings of the Indian Science Congress Association 32(3): 70. 1945. Nirula descreve *Notothylas chaudhurii* Nirula, uma espécie para a Índia, e a Ásia passa ser a o ponto com maior ocorrência do gênero.
- 1977 Udar & Chandra. Geophytology 7: 142. f. 1–11; pl. 1, f. 1–5. Ram Udar inicia um longo trabalho com o gênero descrevendo uma série de espécies na Índia. *Notothylas pandei* Udar & V. Chandra.
- 1979 Udar & Singh. Revue Bryologique et Lichénologique 45: 202. f. 1. 1979. Udar & D. K. Singh. Lindbergia 5: 28. f. 1. 1979. Ram Udar e D. K. Singh descrevem duas espécies para a Índia e esta passa a ser o país com maior ocorrência do gênero; *N. anaporata* Udar & D.K. Singh. *N. pfleidereri* Udar & D.K. Singh.
- 1979 Jiro Hasegawa. Acta Phytotaxonomica et Geobotanica 30: 26. f. 4. 1979. Hasegawa descreve duas espécies para o Japão: *N. depressispora* J. Haseg. *N. temperata* J. Haseg.
- 1980 Udar & Singh. Miscellanea Bryologica et Lichenologica 8: 173. f. 1. 1980. Udar & D.K. Singh homenageiam ao Dr. Daniel Vital ao descreverem *N. vitalii* Udar & D.K. Singh. espécie endêmica do Brasil.
- 1981 Journal of Bryology 11: 451. f. 1. pl. 1. 1981. Udar & D.K. Singh descrevem *N. himalayensis* Udar & D.K. Singh com referência ao Himalaia e *N. khasiana* Udar & D.K. Singh descrita para Shillong, Índia.
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- 1991 A.K. Asthana & S.C. Srivast. Bryophytorum Bibliotheca 42: 106. 1991. Inclusão do subgênero *Notothyloides* que destaca a ausência da columela e quantidade de células na linha de deiscência.
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- Tao Peng e Rui-Liang Zhu descrevem *N. yunnanensis* T. Peng & R.L. Zhu espécie chinesa.
- N. irregularis* Chant. é a primeira descrição para a Tailândia, apesar de haver outras espécies, faz referência a irregularidade das células da cápsula.
- N. frahmii* Chant. foi a última espécie descrita para o gênero, a qual faz homenagem ao Prof. Dr. Jan-Peter Frahm.

6.2. ANNEX II. Table 1. Morphological comparison of Brazilian *Notothyas* species

Characters	<i>N. breutelii</i>	<i>N. granulata</i>	<i>N. javanica</i>	<i>N. orbicularis</i>	<i>N. vermiculata</i>
Dehiscence line	Present	present	absent	present	present
Epidermal cells	strongly thick walled	strongly thick walled	moderately thick walled	strongly thick walled	strongly thick walled
Arrangement of epidermal cells	Regular	regular	irregularly	irregularly	regular
Shape of epidermal cells	rectangular	quadratic to rectangular	quadratic to subrectangular	subquadratic to subrectangular	subquadratic to rectangular
Pseudoelater	always present	always present	mostly desintegrated or absent	Present	present
Spore colour	brown to black	brownish	yellow	yellow	brownish
Ornamentation	baculate	tuberculate	finely vermiculate	vermiculate	strongly vermiculate
Distal face	without a hump-like projection	without a hump-like projection	without a hump-like projection	without a hump-like projection	with a hump-like projection

Proximal face	baculate without central hollow	tuberculate with central hollow	vermiculate without central hollow	vermiculate without central hollow	vermiculate with central hollow
size	(36) 47-51 μm	(25) 30-36 μm	50-62 μm	38-40 μm	(28) 32-38 μm
Columella	well-developed	well-developed	absent	well-developed	well-developed

6.3. ANNEX III. Primers used in this study, daggers (†) are for primers using only for sequencing.

Locus	Primer name	Sequence (5'-3')	Source reference
<i>rbcL</i>	<i>rbcLF</i>	GTCACCACAAACGGARACTAAA GC	Duff et al. (2004)
<i>rbcL</i>	<i>rbcLHR</i>	CTTTCCATACTTCRCAAGCAGC	Duff et al. (2004)
<i>rbcL</i>	<i>rbcL471F†</i>	CAAGGTCCACCTCATGGTA	Duff et al. (2004)
<i>rbcL</i>	<i>rbcL660R†</i>	AACGATCTCTCCAACGCA	Duff et al. (2004)
<i>rbcL</i>	<i>rbcL946R†</i>	ACACGAAAGTGAATACCATG	Duff et al. (2004)
<i>rbcL</i>	<i>rbcL946F†</i>	ACACGAAAGTGAATACCATG	
trnL-F intron	<i>trnC</i>	CGAAATTGGTAGACGCTG	Quandt & Stech (2004)
trnL-F intron	<i>trnD</i>	GGGGGTAGAGGGACTTGAAC	Quandt & Stech (2004)
trnL-F intron	<i>trnDi†</i>	CTTCCATTGAGTCTCTGCACC	Quandt & Stech (2004)
trnL-F spacer	<i>trnD**</i>	GTTCAAGTCCCTCTACCCCC	
trnL-F spacer	<i>trnF</i>	ATTTGAACTGGTGACACGAG	Quandt & Stech (2004)
<i>rbcS</i>	<i>rbcS-F</i>	GTC CGT GGT CGC ATC CTC	Villarreal unpublished
<i>rbcS</i>	<i>rbcS-R</i>	AAG GCT TGT GGA CGA TGA AG	Villarreal unpublished

6.4. ANNEX IV. Morphological data used in the cluster analysis. A. Gametophyte form, B. Presence of pyrenoid, C. Spore color, D. spore ornamentation, E. Conical projection on outer surface, F. Presence of pseudo elater, G. Presence of columela, H. Presence of valva in capsulae, I. Number of cell layer in the valava, J. Depression inner spore, K. Spore wide group, L. Spore long group.

Taxon	code	A	B	C	D	E	F	G	H	I	J	K	L
<i>Notothylas anaporata</i>	nana	2	1	2	2	1	1	1	1	2	0	2	2
<i>Notothylas breutelii</i>	nbre	1	1	2	1	0	1	1	1	2	0	1	1
<i>Notothylas depressispora</i>	ndep	3	1	1	3	1	1	1	1	2	1	1	1
<i>Notothylas dissecta</i>	ndiss	3	1	2	2	1	1	1	1	2	1	1	1
<i>Notothylas flabelata</i>	nfla	1	0	2	2	1	1	0	1	4	0	1	2
<i>Notothylas frahmii</i>	nfra	3	1	1	3	1	0	1	1	2	1	1	1
<i>Notothylas irregularis</i>	nirr	3	1	1	3	1	0	1	1	2	1	1	1
<i>Notothylas kasiana</i>	nkas	3	1	1	3	1	0	0	1	2	0	2	2
<i>Notothylas khasiapii</i>	nkha	3	1	2	2	1	1	0	1	3	0	1	2
<i>Notothylas pandei</i>	npan	2	1	2	3	1	1	1	1	2	1	1	1

<i>Notothylas pflidereri</i>	npfe	2	1	1	3	1	1	0	1	4	0	2	2
<i>Notothylas udari</i>	nuda	3	1	2	3	1	1	1	1	3	0	2	2
<i>Notothylas yunnanensis</i>	nyun	3	1	1	3	0	0	1	0	0	1	1	1
<i>Notothylas temperata</i>	ntem	3	1	2	3	1	1	1	0	0	0	2	2
<i>Notothylas javanica</i>	njav	3	0	1	3	1	0	0	0	0	0	2	2
<i>Notothylas indica</i>	nind	3	1	2	1	1	1	1	1	3	0	2	2
<i>Notothylas nepalensis</i>	nnep	2	0	1	3	0	1	0	1	4	0	1	2
<i>Notothylas himalayensis</i>	nhim	3	0	1	3	0	1	1	1	3	0	2	2
<i>Notothylas galapagensis</i>	ngal	3	1	1	1	0	1	1	1	2	0	1	2
<i>Notothylas orbicularis</i>	norb	3	0	1	3	0	1	1	1	2	0	1	1
<i>Notothylas</i> sp1	nsp1	3	1	2	1	1	1	1	1	2	1	1	1
<i>Notothylas</i> sp2	nsp2	3	1	2	3	1	1	1	1	2	1	1	1

6.5. ANNEX V. Description of the sequences of mark rbcL, of the analysis CI: consistency index and CR: retention index.

Characteristics	Region rbcL
Taxons included	29
Matrix length	600/1300
Variable characters	10
Informative characters	24
Number of trees	10.000
CI	1
RI	1
Replacement model	GRT