Plant and Fungal Systematics 71(2): 365–377, 2026
DOI: https://doi.org/10.35535/pfsyst-2026-0027

ISSN 2544-7459 (print)
ISSN 2657-5000 (online)

New records of *Trypetheliaceae* from Vietnam and the synonymy of *Marcelaria benguelensis* under *Buscalionia cumingii*

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Article info

Received: 20 Dec. 2025
Revision received: 21 Mar. 2026
Accepted: 5 May 2026
Published: 23 Nov. 2026

Associate Editor

Martin Kukwa

Abstract. Nineteen *Trypetheliaceae* species, mainly collected in the Southern Vietnam Lowland Dry Forest, are newly recorded for Vietnam. Five genera, including *Bogoriella*, *Buscalionia* (= *Marcelaria*), *Constrictolumina*, *Macroconstrictolumina* and *Polymeridium*, are reported from the country for the first time. In addition, phylogenetic analyses based on a concatenated mtSSU–nuLSU dataset and morphological assessments were employed to resolve taxonomic uncertainties in *Buscalionia* specimens with ambiguous diagnostic characters. The results support the reduction of *Marcelaria benguelensis* as a synonym of *Buscalionia cumingii* comb. nov. (≡ *Marcelaria cumingii*).

Key words: ascospore number, morphology, Nui Chua National Park, phylogenetic analysis, Ta Kou Nature Reserve, thallus pruinosity

Introduction

Trypetheliaceae is one of the most diverse families of crustose lichen in the tropics. They exhibit high species richness in tropical dry forests, grasslands, and canopy layers of rainforests (Aptroot et al. 2016). During field surveys conducted in Nui Chua National Park and Ta Kou Nature Reserve, located within the Southern Vietnam Lowland Dry Forests ecoregion, we collected hundreds of *Trypetheliaceae* specimens, including several taxa that had not been reported from the country. Among these, numerous specimens belong to the genus widely known as *Marcelaria*, which has never been officially recorded from Vietnam.

Marcelaria has been established since 2013 with three species defined by morphology: *M. benguelensis*, *M. cumingii* and *M. purpurina*. Under the genus *Marcelaria*, *M. benguelensis* and *M. cumingii* closely resemble each other in terms of morphology and distribution (Aptroot et al. 2013). Two species are distributed in Eastern Palaeotropical regions (Aptroot & Lücking 2016). *Marcelaria cumingii* and *M. benguelensis* have been conventionally distinguished by thallus pruinosity, ascospore numbers and minor differences in perithecial and ostiolar sizes (Aptroot et al. 2013; Aptroot & Lücking 2016; Schumm & Aptroot 2021). In the genus revision, the authors highlighted that *M. benguelensis* has yellow pruina on the thallus surface, smaller perithecia and narrower ostioles compared to those of *M. cumingii* which has non-pruinose thallus (Aptroot et al. 2013). The diagnostic couplet has been utilized until now with an additionally important character of asci. Specifically, *M. benguelensis* forms 8-spored asci, different from 2-spored asci of *M. cumingii* (Aptroot et al. 2013; Aptroot & Lücking 2016; Aptroot 2021). However, these reports are inconsistent with the original description of *Trypethelium cumingii*, the basionym of *M. cumingii*, in which Montagne (1845) described the species as having 8-spored asci. Additionally, the illustration of *M. cumingii* provided in the genus revision portrays eight-spored asci, which contradicts the two-spored asci stated in the description (Aptroot et al. 2013). The discrepancies raise questions regarding taxonomic interpretation or possible intraspecific variation within *M. cumingii*. From another perspective, it is hypothesized that the development of pruina on the thallus might be influenced by environmental factors rather than being exclusive to particular species (Büdel & Scheidegger 2008; Wilk & Osyczka 2024). Therefore, the use of thallus pruinosity as a diagnostic feature at the species level in *Marcelaria* and the family *Trypetheliaceae* requires further research. In recent decades, phylogenetic analyses of *Trypetheliaceae* have clarified placements of most genera, as well as numerous species in the family (Nelsen et al. 2014; Lücking et al. 2016; Thiyagaraja et al. 2021), yet the position of

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M. benguelensis and its relationship with *M. cumingii* remain unresolved due to the deficiency of molecular data (Hongsanan et al. 2020).

This study presents molecular analyses of *Marcelaria* aimed at resolving the phylogenetic position of *M. benguelensis*. The name *Marcelaria* was proposed for conservation against *Buscalionia* by Aptroot & Nepi (2017). However, the proposal has been rejected by the Nomenclature Committee for Fungi and the General Committee (May & Lendemer 2023; Wilson 2024). Since then, no studies have addressed the nomenclature consequences of this decision. In this study, the genus is treated as *Buscalionia* and the combination of *Buscalionia cumingii* is proposed. In addition, 19 new records of *Trypetheliaceae* for Vietnam are also reported here.

Materials and methods

Morphology and anatomy

Specimens were examined under a Krüss MSZ5000-IL stereomicroscope and Olympus BX53 compound microscope to observe morphological and anatomical structures, respectively. To study chemistry, ultraviolet light (365 nm) and 10% KOH (K) solution were used to observe the reactions on the thallus surface and on the external and internal parts of pseudostromata and perithecia. Images were captured using a Nikon SMZ25 stereomicroscope equipped with DS-Fi3 microscope camera and an Olympus BX53 compound microscope with an attached DP23 digital camera. Morphological and anatomical characteristics were identified and classified following the family revision by Aptroot and Lücking (2016). After identification using the updated family revision and the world key (Aptroot & Lücking 2016; Aptroot 2021), protologues were consulted for verification. New records were verified through comparison with the family treatment for Vietnam (Joshi et al. 2018) and recently scattered reports.

DNA extraction, PCR and DNA sequencing

Specimens were initially washed under running distilled water to eliminate surface contaminants. Perithecia were carefully separated from the thallus using a sterile razor blade under a stereomicroscope and then soaked in acetone for 30 min to remove polyphenol compounds prior to tissue disruption (Cubero & Crespo 2002). The DNA extraction procedure followed the manufacturer's protocol for the GeneJET Plant Genomic DNA Purification Kit (Thermo Scientific™, USA).

Polymerase Chain Reactions (PCR) were performed with a component of 25 µl consistent with 12.5 µl Master mix 2X (Phu Sa Genomic JSC, Vietnam), 1 µl of each primer, 2 µl DNA template and 8.5 µl nuclease-free water. Two molecular markers, mtSSU and nuLSU, were amplified using primer pairs mrSSU1/mrSSU3R (Zoller et al. 1999) and AL2R/LR6 (Vilgalys & Hester 1990; Mangold et al. 2008), respectively. PCR conditions were as follows: an initial denaturation for 5 min at 95°C; 35 cycles of 30 s at 95°C, either 45 s at 51°C for nuLSU or 45 s at 52°C for mtSSU, and 1 min at 72°C; followed by a final extension at 72°C for 10 min. The PCR products were visualized by running 5 µl of the amplicons on a 1.5% agarose gel. PCR product purification and Sanger sequencing were performed by 1st BASE Laboratories (Malaysia).

Sequence alignment and phylogenetic analyses

Reads were checked on GenBank using BLAST to verify possible contamination. Two regions were aligned independently with available sequences that are labelled as *Marcelaria* and *Trypethelium* from GenBank (Table 1) using the MAFFT online server (Katoh et al. 2019) and manually improved in PhyDE v0.9971 (Müller et al. 2010). *Viridothelium virens* was utilized as an outgroup. The nucleotide substitution model for each region was chosen by jModelTest v2.1.10 (Guindon & Gascuel 2003; Darriba et al. 2012). The selected models were applied for Maximum Likelihood (ML) and Bayesian Inference (BI) analyses. ML was conducted using the IQ-Tree web server (Trifinopoulos et al. 2016), performing 1,000 ultrafast bootstrap replicates to determine branch support. BI analysis was generated in MrBayes v3.2.7 (Ronquist et al. 2012) from the command line, with two independent runs of four chains for 500,000 MCMC generations. Generated trees were visualized and edited using FigTree v1.4.4 and PowerPoint v16.66.1. Bootstrap values ≥75% and posterior probabilities ≥0.95 were considered well-supported and shown on the final tree.

Results

New records of *Trypetheliaceae* for Vietnam

This study documents 19 species of *Trypetheliaceae* newly recorded from Vietnam based on morphological and anatomical assessments, nearly doubling the number of species known from the country. Precise localities for each species were provided together with brief notes where necessary. Five genera – *Bogoriella*, *Buscalionia*, *Constrictolumina*, *Macroconstrictolumina*, and *Polymeridium* – are officially reported for Vietnam here. *Astrothelium ambiguum*, *A. auratum*, *A. subscoria*, and *Constrictolumina esenbeckiana* are documented for the first time in the paleotropics; *Astrothelium nigrorufum* is newly recorded in Southeast Asia.

Astrothelium alboverrucoides Aptroot (Fig. 1A, B)

Notes. Perithecia strongly prominent. Ascospores distinctly muriform, 140–190 × 30–40 µm, 4/ascus, thickened in medium septum. Pycnidia abundant, exposed black, usually grouped.

Specimen examined. VIETNAM. Binh Dinh prov., An Toan Nature Reserve, 14°33′48″N, 108°43′08″E, 895 m, rainforest, epiphyte on upper part of fallen tree, Le 23077 (PHH).

Astrothelium ambiguum (Malme) Aptroot & Lücking (Fig. 1C, D)

Notes. Ascomata solitary or often 2–4 aggregated, erumpent to prominent, different from ascomata pseudostromatic in *A. subdiscretum* which is reported below.

Table 1. Species, voucher information and GenBank accession of the sequences used for phylogenetic analyses. Newly generated sequences marked in bold.

Taxon	Voucher	Isolate	GenBank accession	
			mtSSU	nuLSU
Buscalionia cumingii	Parnmen s.n. (F)	MPN552	KM453854	KM453789
Buscalionia cumingii	RAMK:027993	K11	LC223105	LC223104
Buscalionia cumingii	PHH:0011490	24053	**PZ060163**	**PZ059374**
Buscalionia cumingii	PHH:0011491	24056	**PZ060162**	**PZ059373**
Marcelaria purpuria	Caceres 2009	MPN323A	KM453855	KM453790
Trypethelium eluteriae	Lumbsch 19701a	MPN111	KM453874	GU327726
Trypethelium eluteriae	Nelsen s.n. (F, RAMK)	MPN562	KX215598	KX215685
Trypethelium eluteriae	Nelsen s.n. (F, RAMK)	MPN563	KX215599	KX215686
Trypethelium eluteriae	Parnmen 32	MPN580	KX215601	KX215687
Trypethelium foveolatum	Lücking 30515	MPN351	KM453881	KM453816
Trypethelium inamoenum	Lücking 24125	MPN228	KM453875	KM453810
Trypethelium platyleucostomum	Lücking 30512	MPN349	KM453870	KM453806
Trypethelium platyleucostomum	Lücking 30512	MPN350	KX215602	KX215688
Trypethelium rubroplatystomum	Nelsen s.n. (F)	MPN54	KM453871	KM453807
Trypethelium rubroplatystomum	Nelsen s.n. (F)	MPN65C	KX215603	KX215690
Trypethelium sprengelii	Nelsen 4169	MPN382	KM453867	KM453803
Trypethelium subeluteriae	Lumbsch 19113k	–	DQ328989	–
Trypethelium subeluteriae	Lücking 17611	–	DQ329009	–
Trypethelium subeluteriae	Brazil Nelsen B2	MPN144	–	KX215692
Trypethelium subeluteriae	Nelsen s.n. (F)	MPN49C	KM453882	KM453818
Trypethelium subeluteriae	Gueidan 3043	MPN748	KX215604	KX215693
Trypethelium subeluteriae	Gueidan 3052	MPN749	–	KX215694
Viridothelium virens	Nelsen s.n. (F)	MPN497	KM453884	KM453820

Specimen examined. VIETNAM. Ninh Thuan prov., Nui Chua National Park, Da Hang Lake, 11°42′37.9″N, 109°09′19.4″E, 187 m, 22 Dec. 2023, Le & Ho 23207 (PHH); Binh Tien Stream, 11°46′39″N, 109°10′32E, 155 m, on tree branch growing on rock edge, 25 Dec. 2023, Le & Nguyen 23309b; Cau Gay trail, 11°43′28″N, 109°10′41″E, 158 m, semi-open canopy, on shrubs and plantation trees (Mango), 11 Jul. 2024, Le, Tram & Ho 24022 (PHH); Binh Thuan prov., Ta Kou Nature Reserve, along trekking trail to Ta Kou Peak, 10°48′55″N, 107°53′8″E, 358 m, on tree, 13 Jul. 2024, Le, Tram & Ho 24074 (PHH).

Astrothelium auratum (R.C. Harris) Aptroot & Lücking (Fig. 1E, F)

Notes. Ascospores muriform, 55–75 × 15–23 µm, consistent with the revision (Aptroot & Lücking 2016), but considerably shorter than those reported in original description (~250 × 20–30 µm) by Harris (1984).

Specimen examined. VIETNAM. Binh Thuan prov., Ta Kou Nature Reserve, along trail to Bung Thi Hot Spring, 10°46′54″N, 107°56′2″E, 70 m, on a small tree near the hot stream gate, 14 Jul. 2024, Le & Ho 24100 (PHH).

Astrothelium eustomum (Mont.) Müll. Arg. (Fig. 1G, H)

Specimen examined. VIETNAM. Binh Thuan prov., Ta Kou Nature Reserve, along trekking trail to Ta Kou Peak, 10°48′45″N, 107°53′21″E, 396 m, 13 Jul. 2024, Le, Tram & Ho 24067, 24069 (PHH).

Astrothelium meristosporum (Mont. & Bosch) Aptroot & Lücking (Fig. 2A, B)

Notes. Ostioles are sometimes slightly eccentric instead of persistently apical.

Specimen examined. VIETNAM. Lam Dong prov., Bidoup Nui Ba National Park, behind Giang Ly Station, 12°11′9″N, 108°40′43″E, 1455 m, 28 Feb. 2019, Le (PHH 0009926).

Astrothelium nigrorufum (Makhija & Patw.) Aptroot & Lücking (Fig. 2C, D)

Notes. Pseudostromata internally pigmented, K+ blood red, and ascospores 9–12-septate with oval to rectangular lumina, closely matching the original description and illustration (Makhija & Patwardhan 1993), but disagreeing with the astrothelioid ascospore concept currently applied in *Astrothelium* (Nelsen et al. 2014; Lücking et al. 2016).

Specimen examined. VIETNAM. Binh Thuan prov., Ta Kou Nature Reserve, along trail to Bung Thi Hot Spring, 10°48′21″N, 107°56′52″E, 95 m, 20 Nov. 2023, Le & Vo 23109 (PHH); Ta Kou Station, 10°49′55″N, 107°56′11″E, 52 m, on tree, 12 Jul. 2024, Le, Tram & Ho 24048 (PHH); Ninh Thuan prov., Nui Chua National Park, along trail to Da Vach Lake, 11°44′05″N, 109°13′04″E, 258 m, open canopy, on shrubs, 12 Dec. 2023, Le & Nguyen 23191a (PHH); Binh Tien Stream, 11°46′16″N, 109°10′01″E, 294 m, on shrubs, 25 Dec. 2023, Le & Nguyen 23312 (PHH).

Astrothelium scoria (Fée) Aptroot & Lücking (Fig. 2E, F)

Notes. Ascospores 3-septate, 24–30 × 8.9 –12 µm, slightly larger than previous records (Aptroot & Lücking 2016).

Specimen examined. VIETNAM. Ninh Thuan prov., Nui Chua National Park, Binh Tien Stream, 11°46′16″N, 109°10′01″E, 294 m, 25 Dec. 2023, Le & Nguyen 23311 (PHH); Da Vach Lake, 11°44′5″N, 109°13′11″E, 260 m, open canopy, on woody liana, 10 Jul. 2024, Le & Tram 24021a (PHH).

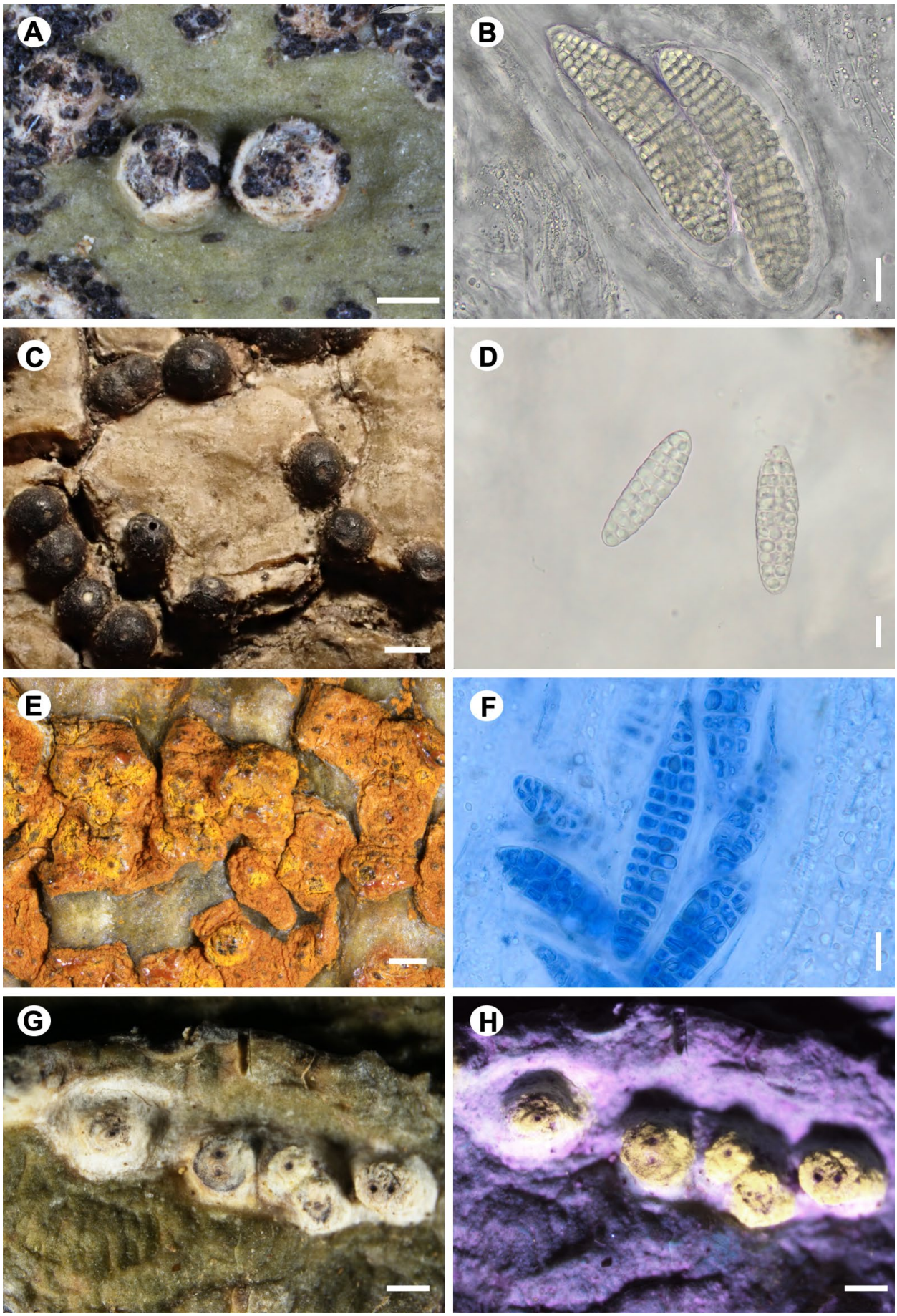

Figure 1. Photographs of A, B – *Astrothelium alboverrucoides* (Le 23077); C, D – *A. ambiguum* (Le & Ho 23207); E, F – *A. auratum* (Le & Ho 24100); G, H – *A. eustomum* in daylight (G) and in 365 nm UV light (H) (Le, Tram & Ho 24069). Scales: A, E, G, H = 0.5 cm; B = 25 µm; D, F = 10 µm. (B, D – water preparation; F – LPCB preparation).

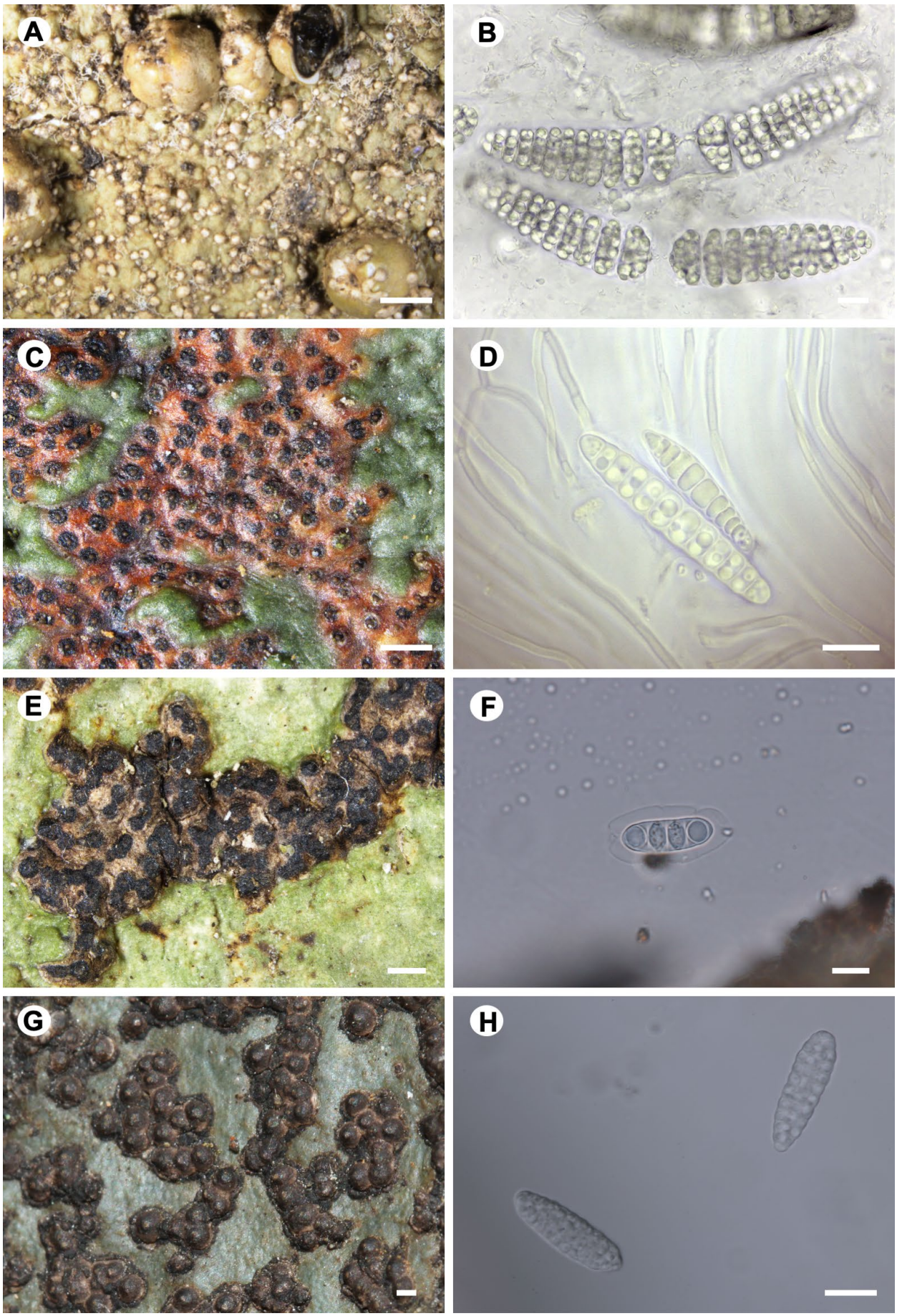

Figure 2. Photographs of A, B – *Astrothelium meristosporum* (PHH 0009926); C, D – *A. nigrorufum* (Le & Vo 23109); E, F – *A. scoria* (Le & Nguyen 23311); G, H – *A. subdiscretum* (Le & Nguyen 23166a). Scales: A = 1 mm; B, H = 20 µm; C, E, G = 0.5 mm; D, F = 10 µm (B, D, F, H – water preparation).

Astrothelium subdiscretum (Nyl.) Aptroot & Lücking (Fig. 2G, H)

Notes. Ascomata aggregate in lines or reticulate, immersed or exposed black from pseudostromata, differing from the solitary to 3–4-aggregated and prominent ascomata in *A. ambiguum*.

Specimen examined. VIETNAM. Ninh Thuan prov., Nui Chua National Park, Vinh Hy Bay, 11°43′36″N, 109°11′27″E, 19 m, along stream, on mango trees, 10 Dec. 2023, Le & Nguyen 23166a (PHH); Da Hang Lake, 11°42′59″N, 109°9′9″E, 356 m, open canopy, on shrubs, 22 Dec. 2023, Le & Ho 23208a (PHH); Binh Thuan prov., Ta Kou Nature Reserve, along trail to Bung Thi hot Stream, 10°48′21″N, 107°56′52″E, 95 m, 14 Jul. 2024, Le & Ho 24089 (PHH).

Astrothelium subscoria Flakus & Aptroot (Fig. 3A, B)

Specimen examined. VIETNAM. Binh Thuan prov., Ta Kou Nature Reserve, along trail to Bung Thi Hot Spring, 10°48′21″N, 107°56′52″E, 95 m, 20 Nov. 2023, Le & Vo 23126 (PHH); Ninh Thuan prov., Nui Chua National Park, Da Vach Lake, 11°44′5″N, 109°13′11″E, 260 m, open canopy, on tree, 10 Jul. 2024, Le, Tram & Ho 24021b (PHH).

Bathelium madreporiforme (Eschw.) Trevis. (Fig. 3C)

Notes. Pseudostromata internally yellow-orange pigment, K+ blood red purplish hue. Hamathecium clear except for the ostiolar region with small droplets. Ascospores muriform, IKI+ weakly purple, 39–68 × 13–17 µm, inconsistent with IKI– and smaller ascospores, which measure 40–55 × 11–17 µm in the revised description (Aptroot & Lücking 2016). The ascospore dimensions slightly overlap with *B. mastoideum* (50–80 × 13–20 µm) – a very similar species aside from ascospore size (Harris 1984; Aptroot & Lücking 2016). However, we assign our specimen to *B. madreporiforme* based on the existence of pseudostromata containing over ten ascomata, in contrast to descriptions by Acharius (1803, 1810), who described *B. mastoideum* as usually producing groups of 1–3 ascomata, with groups exceeding four rarely observed.

Specimen examined. VIETNAM. Binh Thuan prov., Ta Kou Nature Reserve, along trail to Bung Thi Hot Spring, 10°46′24″N, 107°55′52″E, 46 m, on a small tree, 14 Jul. 2024, Le & Ho 24098c (PHH).

Bathelium nigroporum (Makhija & Patw.) Aptroot & Lücking (Fig. 3D)

Notes. Pseudostromata K+ red, agreeing with the original description (Makhija & Patwardhan 1993)

Specimen examined. VIETNAM. Lam Dong prov., Bidoup Nui Ba National Park, 12°10′0″N, 108°40′59″E, 1524 m, 2008 Vo (PHH 0007730).

Bogoriella decipiens (Müll. Arg.) Aptroot & Lücking (Fig. 3E, F)

Specimen examined. VIETNAM. Ninh Thuan prov., Nui Chua National Park, along trail to Da Vach Lake, 11°44′07″N, 109°12′51″E, 209 m, 12 Dec. 2024, Le & Nguyen 23174 (PHH).

Buscalionia cumingii (Mont.) Le & Vo (Fig. 6)

Note. All specimens correspond to the delimitation of species traditionally placed in *Marcelaria benguelensis* particularly in having eight-spored asci (Aptroot et al. 2013; Aptroot & Lücking 2016). However, other diagnostic characters, including thallus pruinosity, the sizes of perithecia and ostioles, vary among specimens, and overlap with those of *M. cumingii* – here treated under *Buscalionia*. In this study, we adopt the name *Buscalionia cumingii* and place *M. benguelensis* in synonymy under *B. cumingii*, based on phylogenetic analyses and morphological evaluations. The taxonomic treatment is detailed in the Taxonomic notes on *Buscalionia* section.

Specimen examined. VIETNAM. Ninh Thuan prov., Nui Chua National Park, Vinh Hy Bay, 11°43′36″N, 109°11′27″E, 19 m, along a stream, on plantation tree, 10 Dec. 2023, Le & Nguyen 23171 (PHH); Da Hang Lake, 11°42′59″N, 109°09′10″E, 356 m, along trekking trail, on tree base, 22 Dec. 2023, Le & Ho 23208b (PHH); trail along Binh Tien Stream, 11°46′39″N, 109°10′32″E, 155 m, on a tree branch growing on rock edge, open canopy, 15 Dec. 2023, Le & Nguyen 23308 (PHH); Binh Thuan prov., Ta Kou Nature Reserve, base of Ta Kou Mountain, 10°48′51″N, 107°55′9″E, 45 m, dry, semi-open canopy, on medium-height trees, 12 Jul. 2024, Le, Nguyen & Ho 24051-24056, 24060 (PHH); Bung Thi hot Stream, 10°46′24″N, 107°55′52″E, 95 m, dry, on small, woody trees, 14 Jul. 2024, Le & Ho 24082a, 24085a, 24091 (PHH); 10°48′22″N, 107°56′52″E, 95 m semi-open canopy, covered by small woody trees, 14 Jul. 2024, Le & Ho 24096 (PHH); 10°46′25″N, 107°55′52″E, 46 m, semi-open canopy, dry, 14 Jul. 2024, Le & Ho 24098a (PHH); Dak Lak prov, Yok Don National Park, 12°51′52.98″N, 107°47′44.34″ E, 192 m, dry deciduous Dipterocarp Forest, partly open, semi-dry, on fallen trunk, 22 Jul. 2024, T.L. Nguyen L24.022 (SGN).

Constrictolumina cinchonae (Ach.) Lücking, M.P. Nelsen & Aptroot (Fig. 3G, H)

Specimen examined. VIETNAM. Ninh Thuan prov., Nui Chua National Park, stone park, 11°37′38″N, 109°09′52″E, 73 m, open canopy, sandy soil covered with scattered shrubs, on a shrub laying on the ground, 22 Dec. 2023, Le & Vo 23139 (PHH).

Constrictolumina esenbeckiana (Fée) Lücking, M.P. Nelsen & Aptroot (Fig. 4A, B)

Notes. Our specimen is consistent with the species concept of *Constrictolumina esenbeckiana*, except for the granular ornamented ascospores when mature. In the young stage, ascospores can be mistaken for the smooth-walled type. The ornamented ascospore was suggested to be a key characteristic used for separating *Macroconstrictolumina* from *Constrictolumina*, following the recent split of the two genera based on phylogenetic analyses (Hongsanan et al. 2020). As *Constrictolumina esenbeckiana* has never been sequenced, the phylogenetic placement of the species within *Trypetheliaceae* cannot be assessed and requires further study.

Specimen examined. VIETNAM. Ninh Thuan prov., Nui Chua National Park, along trail to Da Vach Lake, 11°44′07″N, 109°12′51″E, 209 m, on shrubs, 12 Dec. 2023, Le & Nguyen 23181b (PHH).

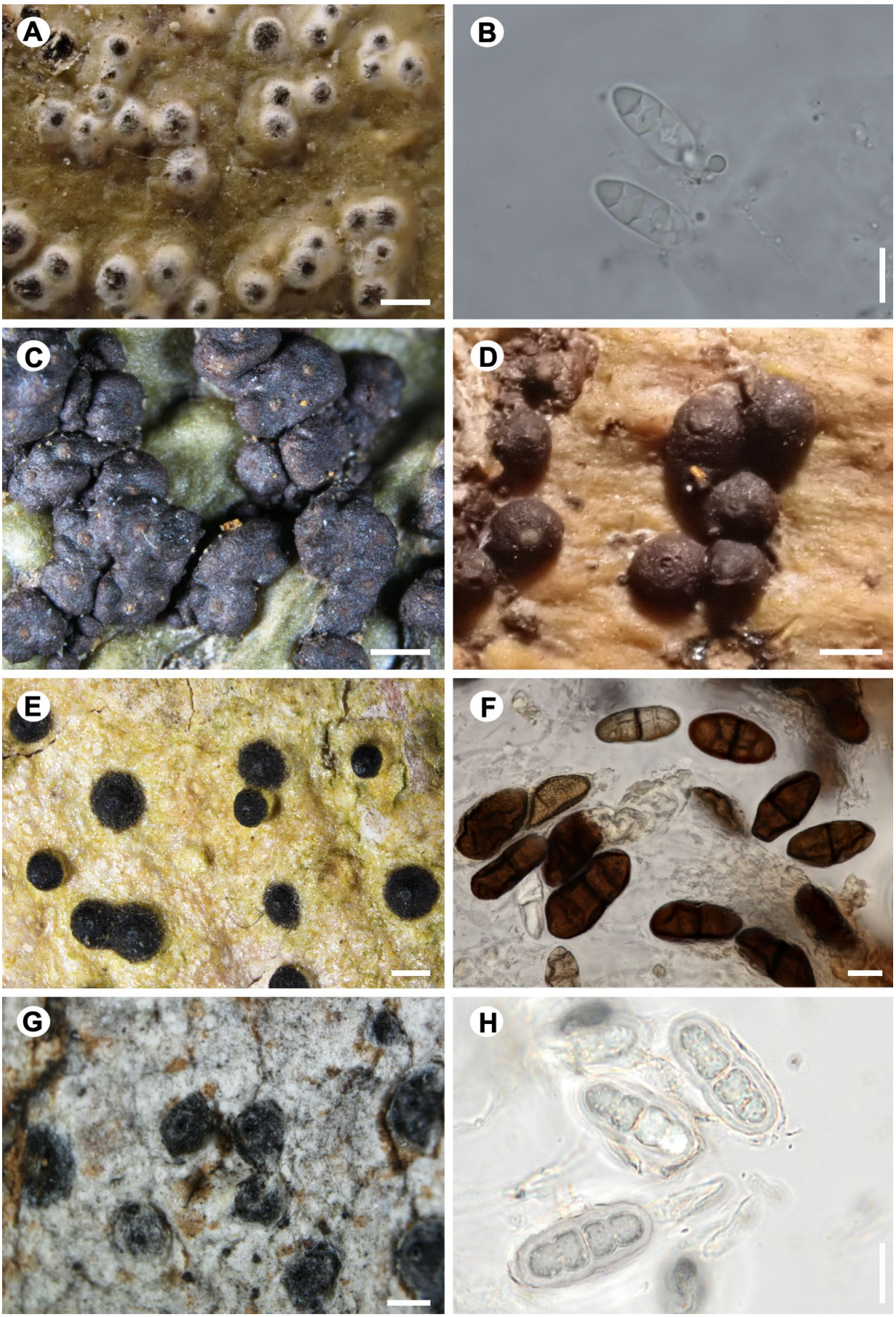

Figure 3. Photographs of A, B – *Astrothelium subscoria* (Le & Vo 23126); C – *Bathelium madreporiforme* (Le & Ho 24098c); D – *B. nigroporum* (PHH 0007730); E, F – *Bogoriella decipiens* (Le & Nguyen 23174); G, H – *Constrictolumina cinchonae* (Le & Vo 23139). Scales: A, C–E, G = 0.5 mm; B, F, H = 10 µm (B, F, H – water preparation).

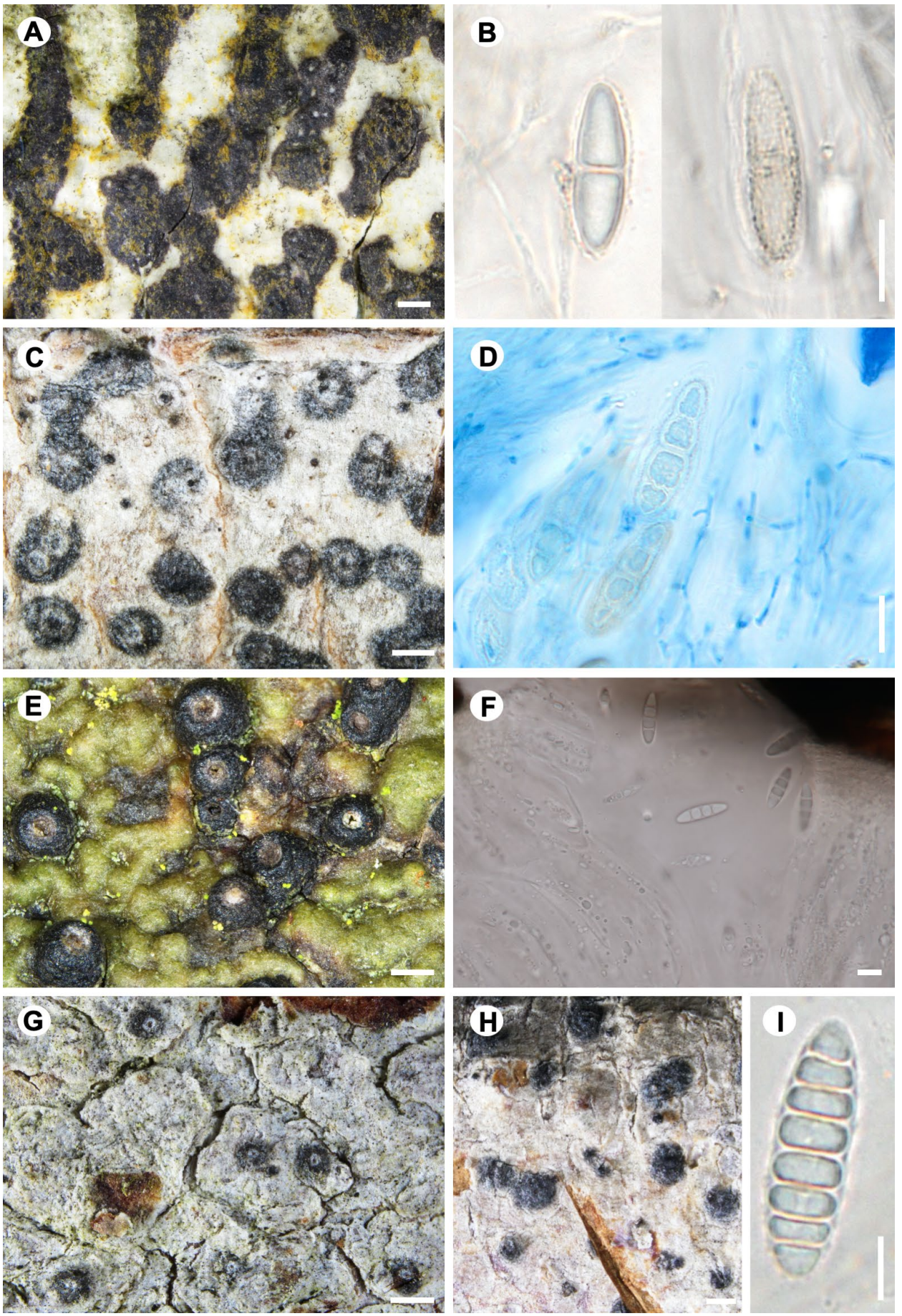

Figure 4. Photographs of A, B – *Constrictolumina esenbeckiana* (Le & Nguyen 23181b); C, D – *Macroconstrictolumina majuscula* (Le & Vo 23132a); E, F – *Nigrovothelium inspersotropicum* (Le & Vo 23116); G – *Polymeridium albocinereum* (Le & vo 23121a); H, I – *P. quinqueseptatum* (Le & Vo 23103b). Scales: A, C, E, G, H = 0.5 mm; B, D, F = 10 µm; I = 5 µm (B, H, I – water preparation; D – LPCB preparation).

Macroconstrictolumina majuscula (Nyl.) Lücking, R. Miranda & Aptroot (Fig. 4C, D)

Specimen examined. VIETNAM. Ninh Thuan prov., Nui Chua National Park, Dinh Ba Slope, 11°37′48″N, 109°09′32″E, 48 m, road side, open canopy covered with scattered shrubs, on shrub, 20 Nov. 2023, Le & Vo 23132a (PHH).

Nigrovothelium inspersotropicum Aptroot & Diederich (Fig. 4E, F)

Specimen examined. VIETNAM. Binh Thuan prov., Ke Ga Cape, 10°42′57″N, 107°59′05″E, 67 m, in a graveyard, open canopy covered by scattered shrubs, sandy soil, on small shrubs, 20 Nov. 2023, Le & Vo 23101 (PHH); Ta Kou Nature Reserve, Bung Thi hot Stream, 10°47′23″N, 107°56′13″E, 75 m, along trail to hot stream, dry, on smooth bark, 20 Nov. 2023, Le & Vo 23116 (PHH); Ta Kou Mountain, sub-area 300, 10°48′51″N, 107°55′9″E, 45 m, on tree along trail 12 Jul. 2024, Le, Nguyen & Ho 24059b (PHH); Ninh Thuan prov., Nui Chua National Park, Da Vach Lake, 11°44′05″N, 109°13′09″E, 258 m, on shrubs along trekking trail, 22 Nov. 2023, Le & Vo 23149 (PHH).

Polymeridium albocinereum (Kremp.) R.C. Harris (Fig. 4G)

Specimen examined. VIETNAM. Binh Thuan prov., Ta Kou Nature Reserve, Bung Thi Hot Spring, 10°46′53″N, 107°56′01″E, 69 m, dry, on a shrub, 20 Nov. 2023 Le & Vo 23121a (PHH).

Polymeridium quinqueseptatum (Nyl.) R.C. Harris (Fig. 4H, I)

Note. Ascospore 5–7-septate, 18–23 × 5–7 µm, different from 8–12-septate, 40–50 × 9–11 µm in *Polymeridium costaricense* (Aptroot & Lücking 2016).

Specimen examined. VIETNAM. Binh Thuan prov., Ke Ga Cape, 10°42′57″N, 107°59′05″E, 67 m, in the graveyard, open canopy covered mostly by scattered shrubs, sandy soil, on shrubs, 20 Nov. 2023, Le & Vo 23103b (PHH).

Taxonomic notes on *Buscalionia*

The genus *Marcelaria* was established in 2013 with *M. purpurina* as the type species (Aptroot et al. 2013). Phylogenetic analyses have confirmed that the genus is monophyletic and forms a sister group to *Trypethelium* (Nelsen et al. 2014; Aptroot & Lücking 2016; Hongsanan et al. 2020; Thiyagaraja et al. 2021). Although the genus name *Marcelaria* has been widely used, it conflicts with the earlier name *Buscalionia* Sambo, which was described in 1940 based on *B. rubra*. Previous studies have shown that the type species of the two genera are conspecific (Daly & Millozza 2007; Aptroot & Nepi 2017; Lücking et al. 2017). The proposal to conserve *Marcelaria* against *Buscalionia* has been rejected by the nomenclature committees (Aptroot & Nepi 2017; May & Lendemer 2023; Wilson 2024). Following the principle of priority, which gives precedence to the earlier name and treats the later name as a synonym, we therefore propose the new combination *Buscalionia cumingii* (Mont.) Le & Vo in this study and adopt this name throughout the manuscript.

Morphology and anatomy

Morphology and anatomy of fifteen specimens examined in the study are consistent with the generic concept previously treated as *Marcelaria*, which is here adopted under the name *Buscalionia* and only the combination for *B. cumingii* is proposed in this study. All samples exhibit thick, yellow pigments covering conspicuously prominent to sessile ascomata and produce eight muriform ascospores per ascus without exception (Fig. 6). According to the taxonomic revision by Aptroot et al. (2013) and recent papers (Aptroot & Lücking 2016; Aptroot 2021), these characters agree with the circumscription of *Marcelaria benguelensis*, and differ from red pigment of *M. purpurina* (*Buscalionia*, comb. ined.) and 2-spored asci of *B. cumingii*. However, the initial publications by Montagne (1845) for *Trypethelium cumingii* and Müller (1885) for *Bathelium benguelense* reported that both species have eight ascospores per ascus. To our knowledge, this inconsistency in the literature suggests that ascospore number is an unreliable character for distinguishing *M. benguelensis* from *B. cumingii*.

In contrast to the above consistent characteristics, morphological observations show the variability in other diagnostic features between specimens. We recorded the variations in perithecium sizes (0.3–1.0 mm), ostiole sizes (ranging from large to narrow), and either presence or absence of pruina on the thallus surface (Fig. 6A–F). The morphological variations overlap from *M. benguelensis* to *B. cumingii*, especially the thallus pruinosity – one of the key features used to distinguish two species in the diagnostic couplet (Aptroot et al. 2013; Aptroot & Lücking 2016; Aptroot 2021). Figures 6A, B display two specimens, No. 24053 and No. 24056, in which the pruina is absent and present on the thallus, respectively, despite occurring in the nearby geographic areas and similar habitats.

Phylogeny

Two genetic markers (mtSSU and nuLSU) were amplified from two representative specimens of *Buscalionia*, comprising No. 24053 with glossy thallus (Fig. 6A) and No. 24056 with yellow-pruinose thallus (Fig. 6B) (Table 1). Both specimens produce 8-spored asci. Single-locus trees revealed a conflict in the *Marcelaria purpurina* placement, yet low bootstrap values indicate weak support (Fig. S1). Therefore, we ignored the conflict and used the concatenated dataset for phylogenetic analyses, applying the model GTR+G according to the Akaike Information Criterion (AIC). The alignment matrix involves 23 sequences (Table 1), with 893 characters: 444 positions from nuLSU and 449 from mtSSU. Maximum Likelihood (ML) and Bayesian Inference (BI) analyses exhibited topologically congruent trees. Hence, we only present the BI phylogenetic tree (Fig. 5).

All sequences of *Buscalionia* and new sequences form a distinct clade separated from the sister group *Trypethelium* (PP = 1.00) (Fig. 5), consistent with previous publications (Aptroot & Lücking 2016; Thiyagaraja et al. 2021). Relationships among species within *Buscalionia*

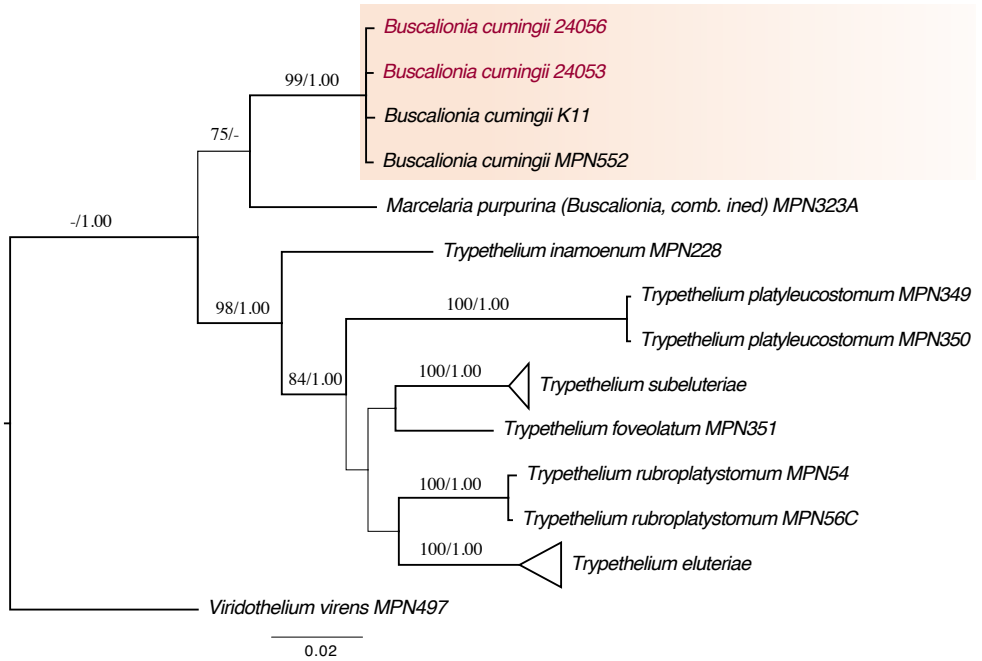

Figure 5. Bayesian tree inferred from a concatenated dataset of mtSSU and nuLSU partial sequences. Bootstrap values ≥75% and posterior probability values ≥0.95 are indicated above the branches.

and *Trypethelium* are well-supported, except for the unresolved clade of *T. subeluteriae*, *T. foveolatum*, *T. rubroplatystomum* and the group of *T. eluteriae*, similar to the results of Aptroot & Lücking (2016). In the single-locus tree mtSSU, *Marcelaria purpurina* is placed as sister to *T. subeluteriae* instead of clustering with other *Buscalionia* sequences, but with low support value (PP = 0.60) (Fig. S1). A similar outcome was generated in the very first molecular study on relationships between genera within *Trypetheliaceae* (Nelsen et al. 2014). The new combined sequences, including No. 23053 and No. 23056 are nested within the same clade and establish a monophyletic group with available DNA of *B. cumingii* (Fig. 5). Bootstrap value (BS = 99) and posterior probability (PP = 1.00) provide robust support for the relationship (Fig. 5). Single-locus analyses using mtSSU (PP = 1.00) and nuLSU (PP = 0.90) persistently provide this result (Fig. S1).

Discussion

Buscalionia cumingii and *Marcelaria benguelensis* share morphological similarities, characterized by pigmentation that forms externally coated prominent to sessile ascomata (Aptroot et al. 2013). The two species have been separated using a diagnostic couplet comprising ascospore numbers, thallus pruinosity, and dimensions of perithecia and ostioles (Aptroot et al. 2013; Aptroot & Lücking 2016; Aptroot 2021). The number of ascospores is considered unreliable to be used as a diagnostic feature due to the inconsistent records between the original description and revised documents (Montagne 1845; Aptroot et al. 2013; Aptroot & Lücking 2016). Meanwhile, it was reported that a yellow-pruinose thallus is usually associated with smaller perithecia and narrower ostioles in *M. benguelensis*, whereas a non-pruinose thallus is associated with larger perithecia and wider ostioles in *B. cumingii* (Aptroot et al. 2013). However, our morphological examination based on specimens from Vietnam supports that the presence of pruina on the thallus, perithecial sizes and ostiolar sizes are independent of one another (Fig. 6A–F).

Newly generated sequences and published data of *Buscalionia cumingii* form separated terminal branches within the strongly supported monophyletic group (Fig. 5). The results show that both studied materials are genetically close, consistent with conspecificity regardless of the presence or absence of thallus pruinosity (Fig. 6A, B). Genetic similarity suggests that the character of thallus pruinosity is unstable for species delimitation here. The phenomenon was also stated for *Marcelaria purpurina*, whose thallus surface is sometimes covered with patches of red pruina (Leighton 1866; Aptroot et al. 2013). This suggests that the variation could represent an intraspecific trait within *Buscalionia* (= *Marcelaria*), and similar variability may occur in other *Trypetheliaceae* taxa. Further sampling and analyses will be necessary to test this hypothesis more thoroughly.

The monophyletic group of new sequences and reference sequences of *Buscalionia cumingii* indicates that examined materials are phylogenetically affiliated with *B. cumingii*, suggesting a need to re-evaluate the morphological concept of the species. Our results show that *B. cumingii* exhibits either yellow-pruinose or non-pruinose thalli, variation in sizes of perithecia and ostioles, and produces 8-spored asci, a character consistent with the original description by Montagne (1845) (Fig. 6). The revised delimitation of *B. cumingii* suggests that diagnostic features traditionally used to separate *B. cumingii* from *Marcelaria benguelensis* may be unstable, providing evidence against the morphological distinction between the two species. Considering molecular evidence and

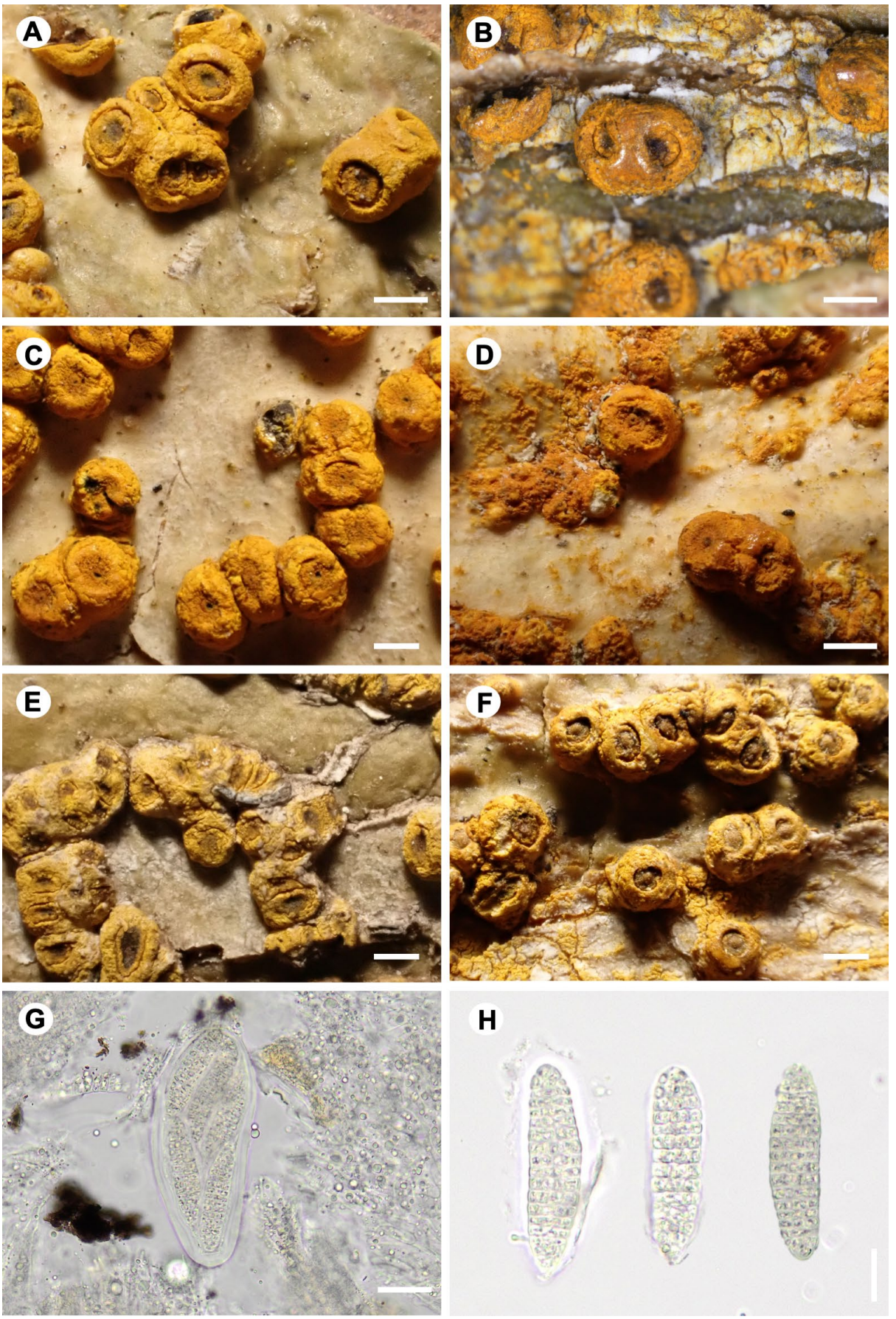

Figure 6. Morphology and anatomy observations of *Buscalionia cumingii* specimens in Vietnam. A, B – large ascomata and wide ostioles with either non-pruinose thallus (A – Le, Tram & Ho 24053) or pruinose thallus (B – Le, Tram & Ho 24056); C, D – large ascomata and narrow ostioles with either non-pruinose thallus (C – Le & Ho 24082a) or pruinose thallus (D – Le, Tram & Ho 24055); E, F – small ascomata and large ostioles with either non-pruinose thallus (E – Le & Ho 24096) or pruinose thallus (F – Le, Tram & Ho 24051); G – ascus 8-spored (Le, Tram & Ho 24056); H – ascospores muriform without thickened medium septum (Le, Tram & Ho 24053). Scales: A–F = 0.5 mm; G, H = 20 µm (G, H – water preparation).

morphological congruence, we therefore propose *M. benguelensis* should be treated as a synonym of *B. cumingii*, relying on currently available data, pending greater geographic sampling. A similar conclusion was also independently suggested in the PhD thesis examining the phylogeny and chemistry of *Trypetheliaceae* in Thailand (Luangsuphabool 2015).

Taxonomic treatment

Buscalionia cumingii (Mont.) Le & Vo, comb. nov. (Fig. 6)

MycoBank MB 865885

Basionym: *Trypethelium cumingii* Mont., London J. Bot. 4: 5. 1845.

Type: Philippines, Cuming s.n. (BM – syntypes, image seen).

≡ *Bathelium cumingii* (Mont.) Trevis., Flora, Regensburg 44: 21. 1861. ≡ *Laurera cumingii* (Mont.) Zahlbr., Cat. Lich. Univers. 1: 503. 1922. ≡ *Marcelaria cumingii* (Mont.) Aptroot, Nelsen & Parnmen, Glalia 5(2): 6. 2013.

= *Bathelium benguelense* Müll. Arg., Flora, Regensburg 68(12): 256. 1885. ≡ *Laurera benguelensis* (Müll. Arg.) Zahlbr., Cat. Lich. Univers. 1: 503. 1922. ≡ *Marcelaria benguelensis* (Müll. Arg.) Aptroot, Nelsen & Parnmen, Glalia 5(2): 6. 2013., syn. nov.

Type: India, Kuru 173 (G – lectotype, image seen).

Description. Thallus corticate, olive green, glossy, smooth to uneven, non-pruinose to yellow-pruinose. Pseudostromata absent. Ascomata abundant, sessile, solitary or aggregate to confluent with separate ostioles, variation in size, 0.3–1 mm, flat on top, usually splitting between the inner wall and the surrounding tissue, covered by thick, yellow-orange pigment. Ostioles apical, broad, sometimes narrow, dark brown to black. Hamathecium heavily inspersed, branched and anastomosing, embedded in a gelatinous matrix, IKI–. Asci 8-spored, clavate, bitunicate, IKI–. Ascospores hyaline, ellipsoid to fusiform, without thickened medium septum, densely muriform, 50–70 × 13–18 µm, IKI–, surrounded by a gelatinous sheath.

Chemistry. Thallus K+ purple in regions covered by pigmented pruina. Perithecia UV+ red orange, K+ blood red purplish hue.

Notes. We observed 8-spored asci for all gathered specimens, consistent with the original description by Montagne (1845).

Distribution. Eastern palaeotropical (India, Cambodia, Myanmar, Thailand, The Philippines and Vietnam).

Acknowledgement

We would like to thank Nui Chua National Park and Ta Kou Nature Reserve for granting collection permissions, and the Center for Ecology and Resources/Institute of Advanced Technology for providing access to laboratory facilities. We are grateful to Prof. André Aptroot for guidance on identification and to Ms. Luong Thien Tam and Dr. Sanna Huttunen for advice on phylogeny analyses. We also thank Dr. Tran Triet and Dr. Nguyen Phi Nga for partially supporting the travel expenses to Nui Chua National Park, and to Prof. Jae-Seoun Hur for travel expenses to An Toan Nature Reserve. Assistance during fieldwork provided by Ms. Ngan and Mr. Thanh is gratefully acknowledged.

Supplementary electronic materials

Figure S1. Bayesian phylogenetic trees inferred using (A) mtSSU and (B) nuLSU sequence datasets. Download file

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