Two new species of *Mesospora* (Ralfsiales, Phaeophyceae) from the subtropical Indo-Pacific region

**SZE-WAN POONG**¹, PHAIk-EEM LIM,¹,², HAJI SUNARPI,³, JOHN A. WEST,⁴, KATHY ANN MILLER,⁵, WENDY A. NELSON,⁶ AND HIROSHI KAWAI⁷

¹Institute of Ocean and Earth Sciences, University of Malaya, 50603 Kuala Lumpur, Malaysia
²Institute of Biological Sciences, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia
³Faculty of Mathematics and Natural Sciences, Mataram University, Lombok-NTB, Indonesia
⁴School of Biosciences 2, University of Melbourne, Victoria 3010, Australia
⁵University Herbarium, 1001 Valley Life Sciences Building 2465, University of California, Berkeley, CA 94720, USA
⁶National Institute of Water and Atmospheric Research, Wellington, New Zealand
⁷Kobe University Research Center for Inland Seas, Rokkodai, Kobe 657-8501, Japan

**ABSTRACT:** The marine crustose brown algal genus *Mesospora* is poorly known despite its wide occurrence in tropical and warm temperate coastal areas. Taxonomic studies on *Mesospora* are largely dependent on the presence of reproductive structures due to its simple thallus morphology and vegetative anatomy. Increased sampling and the combination of molecular and morpho-anatomical studies have revealed new species. *Mesospora indopacifica* sp. nov. and *Mesospora lombokensis* sp. nov. are described based on specimens collected from the subtropical Indo-Pacific. *Mesospora indopacifica* is distinguished by a closely packed basal portion of erect filaments, biseriate plurangia capped by two or three prominent sterile cells and unangia borne on one- or two-celled stalks lateral to the middle of the erect filaments. This new species was found on the east coast of peninsular Malaysia, eastern Australia and Lombok Island, Indonesia. *Mesospora lombokensis* is characterised by tightly adherent erect filaments, biseriate plurangia terminated by one sterile cell and unangia borne on one- to three-celled stalks lateral to the middle of the erect filaments. This species appears to be endemic to Lombok Island, Indonesia. Phylogenetic analyses using plastid *rbcL* and mitochondrial *cox1-5′* genes indicate that both *M. indopacifica* and *M. lombokensis* are genetically distinct from other species of the genus. This study also provides the first *rbcL* sequences of *Mesospora pangoensis* (holotype), *Hapalospongidion gelatinosum* (syntype), *Hapalospongidion saxigenum* and *Basispora africana*.

**KEY WORDS:** Basispora, cox1, Hapalospongidion, Mesospora pangoensis, rbcL

**INTRODUCTION**

Species in the crustose brown algal genus *Mesospora* are incompletely known because of the lack of diagnostic morphological characters for species delimitation, inadequate information on previously described species, confusing variation in terminology related to the position of reproductive structures (León-Alvarez & Norris 2005), a paucity of type material for DNA sequence analysis and in some cases the lack of proper documentation and selection of type specimens when descriptions were published in earlier works. *Mesospora* was established based on *Mesospora schmidii* Weber–van Bosse (1911) from Indonesia. This crustose brown algal genus is generally characterised by the following features: epilithic, mucilaginous and pseudoparenchymatous thallus wholly adherent to the substratum; loosely adherent erect filaments arising from a basal disc of prostrate filaments; intercalary plurangia inserted near the apex of erect filaments and stalked unangia borne laterally at the base of erect filaments (Weber–van Bosse 1913).

Species of *Mesospora* are typically distributed in tropical to warm temperate coastal areas, mostly in the Pacific (e.g. Børgesen 1924; Setchell & Gardner 1937; West & Calum pong 1996) with one report of the type species from the Indian Ocean (Krishnamurthy & Baluswami 1986). *Mesospora macrocarpa* (Feldmann) den Hartog is the only species reported from the Mediterranean Sea (Feldmann 1931, 1937; den Hartog 1968). Six species and one variety are currently recognised, excluding three undescribed species reported by Lim et al. (2007). Five of the species of *Mesospora* were first described on the basis of morphological observations, while *Mesospora elongata* Poong, Lim & Phang was discovered by analysing the plastid large subunit of RuBisCo (*rbcL*) and mitochondrial cytochrome *c* oxidase subunit 1 gene (*cox1-5′*) sequences in addition to morphological observations (Poong et al. 2013). Only three of the six species of *Mesospora* have been genetically analysed (Poong et al. 2014). Species of *Mesospora* are morpho-anatomically distinguished based on a combination of vegetative characters including number of cells per erect filament and thickness of the basal layer and, more importantly, reproductive features such as the position of the unangia, the number of unangial stalk cells and the number of sterile cells terminating the plurangia (Poong et al. 2013).

The family of Mesosporaceae, which included *Hapalospongidion*, Basispora and Mesospora, is characterised by having free erect filaments loosely adjoined in a mucilaginous matrix, intercalary plurangia and unangia arising terminally on a stalk or on a vegetative filament (Tanaka & Chihara 1982). *Hapalospongidion* was established based on

* Corresponding author (phaikeem@um.edu.my).
DOI: 10.2216/16-142.1
© 2017 International Phycological Society
Hapalospongidion gelatinosum Saunders (1899) from Pacific Grove, California, USA. This genus is characterised by its cushion-like mucilaginous thallus (apalos meaning soft; spongios meaning ‘a sponge’ in Greek) composed of long and mostly unbranched erect filaments arising from a basal plate, plurilocular reproductive structures borne near the apex of erect filaments and uniocular reproductive structures arising from the transformation of terminal cells of the shorter erect filaments (Saunders 1899). John & Lawson (1974) erected the genus Basispora based on Basispora africana John & Lawson, which is characterised by unangia arising terminally on long stalks of (4) 6–10 (15) cells from near the base of simple, often assurgent, laterally free vegetative filaments. Poong (2014) presented a detailed review of the three closely related genera. Molecular sequence data were previously unavailable for Basispora; whereas, the internal transcribed spacer (ITS) region of rDNA and 28S rDNA data were generated for Hapalospongidion saxigenum Lindauer in a study by Buchanan (2005). Mesospora is currently treated as a synonym of Hapalospongidion (Womersley 1987; Silva et al. 1996), despite varying opinions regarding the relationship between Hapalospongidion, Mesospora and Basispora (John & Lawson 1974; León-Alvarez & González-González 1993; Rull Lluch 2002; Parente et al. 2006). At this stage, we reserve our conclusions on the appropriate taxonomic treatment for these genera until detailed phylogenetic analyses on more authentic specimens are available (see Discussion).

In this study, rbcL and cox1-5′ gene sequences were analysed in tandem with morpho-anatomical observations to examine the diversity and, to a lesser extent, the biogeography, of Mesospora collected from several locations in the subtropical Indo-Pacific. Here we describe two previously unidentified species as Mesospora indopacifica sp. nov. and Mesospora lombokensis sp. nov.

MATERIAL AND METHODS
Specimens of crustose brown algae attached to their substrata were collected from the littoral zones of the east coast of Peninsular Malaysia; Lombok Island, Indonesia and eastern Australia (Table S1) and air-dried prior to desiccation in silica gel. The holotype specimen of Ralfsia pangoensis Setchell (= Mesospora pangoensis (Setchell) Chihara & Tanaka; UC221298; No. 1001 of Setchell’s collection) deposited in the University Herbarium, University of California, Berkeley (UC), was used for DNA extraction and morphological observation. A syntype specimen of H. gelatinosum (No. 39-80 of the Marine Algae of the Monterey Peninsula’s collection by G. J. Hollenberg from Carmel Beach, California, USA) deposited at the G. M. Smith Herbarium of the Hopkins Marine Station in Pacific Grove, California, USA, was examined. A specimen of Hapalospongidion saxigenum newly collected from its type locality, Stewart Island, New Zealand, was also included in this study. Vouchers of newly collected specimens were deposited in the University of Malaya Seaweeds and Seagrasses Herbarium (KLU) unless otherwise stated. Squash preparations of the specimens mounted on glass slides were observed under light microscope. Photomicrographs were taken using a DP72 digital camera attached to a BX51 microscope (Olympus, Tokyo, Japan).

Genomic DNA extraction and polymerase chain reaction (PCR) amplification for both rbcL and cox1-5′ gene regions were as described in Poong et al. (2013, 2014). Preventive measures were taken to minimize contamination in the handling of the archival samples of Mesospora pangoensis, Basispora africana and Hapalospongidion gelatinosum. This included incorporation of DNA extraction and amplification controls and the use of newly purchased and unopened consumables and reagents. Primers used for amplification and sequencing included: rbcL-rbcFO, rbcR2 (Kawai & Sasaki 2004); PRBF3, PRBR3 (Kogame et al. 1999); RspF2, RspR2 (Poong et al. 2014); RalR952 (Lim et al. 2007); cox1-5′-GazF2, GazR2 (Lane et al. 2007); 117F, 784R (Bittner et al. 2008) and L, H (Folmer et al. 1994). The PCR amplicons were screened for correct length by gel electrophoresis. The target DNA fragments were purified using a LaboPass PCR Purification and Gel Extraction kit (Cosmo Tech, Seoul, South Korea), and gene sequencing was performed by 1st BASE (Selangor, Malaysia). Newly determined rbcL and cox1-5′ sequences were deposited in GenBank (Table S1). Additional sequences included in the phylogenetic analyses (Figs 1, 2) were downloaded from GenBank. Raw sequence data were assembled and edited in ChromasPro v1.42 (2008). Consensus sequences were preliminarily aligned in ClustalX v2.0.8 (Larkin et al. 2007) and later manually trimmed in Bioedit v7.0.9.0 (Hall 1999). Two alignments using individual data sets of rbcL and cox1-5′ were used for the construction of phylogenetic trees. Representatives of the sister families of Mesosporaceae (Lim et al. 2007; Poong et al. 2014) were employed as outgroups to root the trees: Analipus japonicus (Harvey) Wynne and Ralfsia fungiformis (Gunnerus) Setchell & Gardner (Ralfsiaceae); and Neoralfsia expansa (J.Agardh) Lim & Kawai ex Cormaci & Furnari (Neoralfsiaceae).

Maximum parsimony (MP) analyses were executed via PAUP* v4.0b10 (Swofford 2001) using heuristic search with 100 random sequence addition replicates and a tree bisection and reconnection (TBR) branch swapping algorithm. Gaps in the alignment were treated as missing data. All characters were treated as unordered and equally weighted, the Multrees option active and branches with a maximum length of zero collapsed to yield polytomies. Robustness of the trees was determined by computing bootstrap percentage (BP) with 1000 pseudoreplicates using one random taxon addition under the heuristic search method with TBR swapping. The best-fit model of molecular evolution for each data set was deduced from the corrected Akaike information criterion.
and Bayesian information criterion implemented in Kakusan v3 (Tanabe 2007) for maximum-likelihood (ML) and Bayesian inference (BI) analyses, respectively. ML phylogenetic trees were inferred using Treefinder v October 2008 (Jobb et al. 2004) with BP computed from 1000 resamplings to estimate the confidence limits of individual clades. BI phylogenies were estimated with MrBayes v3.1.2 (Ronquist & Huelsenbeck 2003). Two parallel runs were performed using four chains of Markov chain Monte Carlo for 2,000,000 generations, and trees in each chain were sampled every 100th generation. The plot of generation vs log likelihood was inspected with Tracer v1.5 (Tracer 2009) after the run to ensure that stability was reached. The first 2000 trees were discarded as burn-in based on the stationarity of log likelihood values in the first 100,000 generations. The monophyly of Mesospora and each species clade was moderate to fully supported; although, the relationships among species of Mesospora were poorly resolved. Two clades corresponding to undescribed taxa based morphology are presented here as new species: M. indopacific a sp. nov. and M. lombokensis sp.

RESULTS

Molecular phylogenetic analyses

The aligned \textit{rbcL} data set for 24 taxa was 1339 bp in length; the \textit{cox1-5′} alignment for 21 taxa was 653 bp in length. All sequences were unambiguously aligned, and no gaps were present. The newly determined \textit{rbcL} sequence lengths for Mesospora pangoensis, \textit{Hapalospondion gelatinosum}, Basi- spora africana and \textit{Hapalospondion saxigenum} were 1189 bp, 1365 bp, 1334 bp and 1081 bp, respectively. The \textit{rbcL} sequence for \textit{H. gelatinosum} is filled with gaps, indicating DNA degradation due to the aged condition of the specimen and the very limited DNA available for PCR amplification. Amplification of the \textit{cox1} gene was not successful for the \textit{Hapalospondion} and \textit{Basispora} samples despite repeated attempts. Using all three methods of phylogenetic inference (ML, MP, BI), the phylogenetic trees from both datasets were concordant for all well-supported nodes; the ML trees were depicted with support (Figs 1, 2).
nov. Mesospora pangoensis and Mesospora schmidtii were resolved as closely related sister taxa. Hapalospongidion gelatinosum, Hapalospongidion saxigenum and Basispora africana were nested in an unsupported clade that was separate from species of Mesospora (Fig. 1).

Taxonomic descriptions of new species

Mesospora indopacifica S.-W.Poong, P.-E.Lim & S.-M.Phang sp. nov.
Figs 3–6

DESCRIPTION: Thallus crustose, epilithic, mucilaginous upon contact with water, under surface tightly adherent to the substratum without rhizoids and composed of erect filaments arising from a two- to three-layered basal plate; the basal portion of erect filaments is closely packed; whereas, the terminal portion is more loosely associated; erect filaments less than 20 cells long; unangia borne terminally on one- or two-celled stalks, lateral to the middle of the parent filament and surrounding erect filaments; plurangia at first uniseriate, later biseriate at maturity, inserted near the apex of the erect filaments and capped by two or three prominent sterile cells; rbcL sequence = GenBank accession KP689604 and cox1-5’ sequence = KP689621 distinct from other genetically analysed species of Mesospora.

HOLOTYPE: PSM12212; collected by S.-W. Poong and P.-E. Lim on 8 June 2010; deposited in University of Malaya Seaweeds and Seagrasses Herbarium, Kuala Lumpur, Malaysia (KLU); with plurangia.

TYPE LOCALITY: Lendang Guar (08°27.719’S, 116°02.164’E); epilithic, collected at the littoral zone; Lombok Island, Indonesia.

ETYMOLOGY: This species is named after its geographical distribution in the Indo-Pacific region.

OTHER SPECIMENS EXAMINED: Malaysia – PSM12319, PSM 12320, PSM 12321 and PSM 12323, collected 17 February 2012, Pantai Kemasik, Terengganu; PSM 12324 and PSM 12328, collected 16 February 2012, Pantai Chendering, Terengganu; Australia – PSM 12790, PSM12791, PSM 12792 (collected 18 October 2013) and PSM 12822 (collected 15 February 2014) from Sunshine Beach, Queensland, Australia.

MORPHOLOGY AND ANATOMY: The thallus was thin and epilithic, greenish to dark brown, sometimes with an eroded centre; outline was initially circular, it later became irregular and often confluent with surrounding thalli (Fig. 3). The thallus (especially fertile parts) was mucilaginous upon contact with water and closely attached to the substratum without rhizoids. Erect filaments arose from a basal plate of two to three horizontal cell layers, approximately 16 to 26 μm thick, with cells measuring 7.3–25.1 μm wide and 3.4–10.9 μm long. The erect filaments in the lower half of the thallus were closely packed (Fig. 4), requiring more pressure to separate when compared to Mesospora schmidtii and Mesospora elongata. The erect filaments were 68 to 220 μm long and consisted of 6–18 cells; the cells were 3.2–12.2 μm broad and 5.7–20.4 μm long, with a length to diameter ratio of 0.6:1 to 3.5:1.

Plurangia and unangia were found on separate thalli. The plurangia were initially uniseriate and became biseriate when matured. Young and mature plurangia were capped by two or three (seldom one) sterile terminal cells, which were more prominent at maturity (Fig. 5). The plurangia are believed to have developed from...
the swelling and elongation of cells near the distal end of vegetative filaments, followed by horizontal partitioning and longitudinal division into four cells each, as described by Weber–van Bosse (1913) for *Mesospora schmidtii*. The plurangia measured 7.7–12.8 μm in diameter and 27.5–59.2 μm in length. Unangia were borne on one- or two-celled stalks lateral to the middle of the erect filaments (Fig. 6). The unangia were 18.8–37.4 μm broad and 37.1–70.7 μm long, with a length to diameter ratio of 1.4:1 to 2.5:1.

**HABITAT, DISTRIBUTION AND SEASONALITY:** The thalli of *Mesospora indopacifica* grew on rocks in the littoral zones. This species was collected from Lendang Guar, off the west coast of Lombok Island in June. On the east coast of Peninsular Malaysia, this species was found in February at Pantai Kemasik and Pantai Chendering, Terengganu. The thallus of *M. indopacifica* was sometimes confluent with thalli of other species of *Mesospora* such as *Mesospora negrosensis* J.West & H.Calumpong. In eastern Australia, the thalli of *M. indopacifica* were collected in February and October at Sunshine Beach, Queensland. Specimens collected from Peninsular Malaysia were sterile; whereas, those from Lombok Island, Indonesia and Sunshine Beach, Australia had either plurangia or unangia.

**Mesospora lombokensis** S.-W.Poong, P.-E.Lim & S.-M.Phang *sp. nov.*

**DESCRIPTION:** Thallus thin, light to dark brown, forming a disc-shaped crust on rocks, later tending to be confluent with surrounding thalli, firmly attached to substratum without rhizoids, mucilaginous upon contact with water; erect filaments laterally cohesive except in the reproductive portions, consisting of approximately 20 cells, curved and assurgent from a thin, one- to three-layered basal plate; plurangia initially uniseriate, later biseriate and capped by a sterile terminal cell at maturity; unangia borne terminally on one- to three-celled stalks lateral to the middle of the parent filament and surrounding filaments; *rbcL* sequence = GenBank accession KP689614 and *cox1-5* sequence = KP689631 distinct from other genetically analysed species of *Mesospora*. 

**Figs 3–6. Mesospora indopacifica* sp. nov.**

**Fig. 3.** Crusts of the holotype on its substratum (voucher number: PSM 12212).
**Fig. 4.** Squash preparation showing a young and developing vegetative thallus ( voucher number: PSM 12319). Scale bar = 50 μm.
**Fig. 5.** Plurangia capped by two or three prominent sterile terminal cells (voucher number: PSM 12212). Scale bar = 50 μm.
**Fig. 6.** Unangia borne terminally on one- or two-celled stalks, (indicated with arrows) lateral to the middle of the erect filaments (voucher number: PSM 12791). Scale bar = 50 μm.
HOLOTYPE: PSM12239; collected by S.-W. Poong and P.-E. Lim on 12 June 2010; deposited in University of Malaya Seaweeds and Seagrasses Herbarium, Kuala Lumpur, Malaysia (KLU); with unangia and plurangia.

TYPE LOCALITY: Labuhan Pandan (08°29.203'S, 116°39.695'E); epilithic in the littoral zone; Lombok Island, Indonesia.

ETYMOLOGY: This species is named after its type locality.

OTHER SPECIMENS EXAMINED: PSM12218; collected 8 June 2010 from Batu Layar, Lombok Island, Indonesia; PSM12240, PSM12241, PSM12242 (collected 12 June 2010) from Labuhan Pandan, Lombok Island, Indonesia.

MORPHOLOGY AND ANATOMY: The thallus was thin, epilithic, light to dark brown, sometimes with an eroded centre and was attached firmly to the substratum without the presence of rhizoids (Fig. 7). The thallus was initially circular in outline but later tends to be indefinite and sometimes overlapped other thalli. The thallus was composed of prostrate filaments from which upwardly curving and laterally cohesive filaments arose. The basal plate was thin, approximately 10–12 μm in thickness, with cells measuring three to

Figs 7–12. *Mesospora lombokensis* sp. nov.

Fig. 7. Crusts of the holotype on its substratum (voucher number: PSM 12239)

Fig. 8. Squash preparation showing a three-tiered structure consisting of the plurangia, the closely adherent and assurgent erect filaments and the basal layer at low (×4) magnification (voucher number: PSM 12239). Scale bar = 500 μm.

Fig. 9. Uniseriate and biseriate plurangia terminated by a single sterile cell near the apex of erect filaments with a disrupted basal layer (voucher number: PSM 12239). Scale bar = 50 μm.

Fig. 10. Squash preparation showing the structures mentioned in Fig. 8 at a higher (×40) magnification (voucher number: PSM 12241). Scale bar = 50 μm.

Fig. 11. A unangium borne terminally on a three-celled stalk (indicated by arrow), lateral to the middle of the erect filaments with disrupted basal layer (voucher number: PSM 12239). Scale bar = 50 μm.

Fig. 12. A unangium borne terminally on a three-celled stalk, lateral to the middle of the erect filaments with partially disrupted basal layer (voucher number: PSM 12239). Scale bar = 50 μm.
four times as wide as long. Plurangia and unangia were found on separate thalli. The thallus had three layers that were clearly seen in thalli bearing plurangia (Fig. 8) and was somewhat reminiscent of the delineation between cortical and median layers in *Neoralfsia expansa*.

Plurangial thalli and unangial thalli developed differently. During the development of plurangia, the uppermost portion of the erect filaments differentiated into plurilocular structures capped by sterile terminal cells, this corresponds to the first layer. The plurangia were at first uniseriate and later biseriate at maturity, measuring 14.9–58.1 μm long and 4.8–12.0 μm wide (Fig. 9). The central layer was composed of cells directly below the plurangia, which were 4.7–8.4 μm wide and 5.3–13.6 μm long (length to diameter ratio of 0.8:1 to 2.5:1). Cells of the lower layer were more elongated measuring 6.1–11.7 μm in diameter and 10.9–31.2 μm in length (length to diameter ratio of 1.3:1 to 4.9:1) and were sometimes branched (Fig. 10). The distinction between the central and bottom layers may not be apparent, especially when the bottom layer and basal plate were disrupted during the squash preparation process. In contrast, during the development of unangia, the distal cells of the erect filaments became more elongated measuring 3.4–8.3 μm broad and 5.1–15.5 μm long (length to diameter ratio of 0.8:1 to 3:2:1) so that each filament was club-shaped. Cells composing the lower half of the erect filament were 4.2–9.4 μm broad and 5.7–23.7 μm long (length to diameter ratio of 0.8:1 to 2.7:1). Unangia-bearing thalli did not appear three-layered, and the lower half portion of the erect filaments were often detached during the squashing process (Fig. 11).

Unangia were borne terminally on one- to three-celled stalks, inserted in the middle portion of the erect filaments (Fig. 12). The unangia were 9.1–33.8 μm in diameter and 29.9–88.3 μm in length (length to diameter ratio of 2.1:1 to 4:1:1). This species resembles *Mesospora negrosensis* but is distinguished by its smaller plurangia.

**HABITAT, DISTRIBUTION AND SEASONALITY:** *Mesospora lombokensis* was found growing on rocks in the littoral zone. It was collected at two sites, Labuhan Pandan and Batu Layar, Lombok Island, Indonesia, in June. *Mesospora lombokensis* was sometimes found with a species tentatively identified as *Diplura*.

**Morpho-anatomical observations of Mesospora pangoensis, Basipsora africana, Hapalospondion gelatinosum and Hapalospondion saxigenum**

*MESOSPORA PANGOENIS* (Fig. 13): The thallus was thin and epilithic, dark brown to black, and firmly adherent to the substratum without rhizoids. Erect filaments were laterally free, held together by mucilaginous material, consisting of less than 20 cells, 145–210 μm in length and arose from a basal plate of generally two cells (12–18 μm) thick. Cells of the basal plate were 7–10 μm long and 11–18 μm broad, with a length to diameter ratio of 0.4:1 to 0.8:1. Cells at the distal end of the erect filaments tend to be cylindrical, while cells at the lower part were globose. The cells were 8–14 μm long and 4–11 μm broad, with a length to diameter ratio of 0.9:1.0 to 2:0:1.0. Unangia were inserted terminally on stalks of two or three cells, lateral to the middle of the erect filaments. The unangia were 70–150 μm long and 20–60 μm broad. Plurangia were initially uniseriate, later biseriate when matured. Young and mature plurangia were both capped by one to three (generally two) sterile terminal cells, which were more prominent at maturity. The plurangia measured 9–12 μm in diameter and 25–38 μm in length.

*BASEIPSORA AFRICANA* (Fig. 14): The thallus was dark brown to black. Layers of prostrate filaments were about 12–17 μm thick, giving rise to loosely adherent erect filaments 340–480 μm in length and comprised of approximately 40 cells. Cells of the erect filaments were 6–20 μm long and 2–7 μm broad; with a length to diameter ratio of 1.2:1 to 7:1.1. Measurements of cell number and size may not be accurate due to the somewhat deteriorated state of the specimen. No reproductive structures were observed.

**HAPALOSPONDION GELATINOSUM** (Figs 15–18): The erect filaments were laterally free, arising from a thin basal layer (not clearly seen in the syntype and Hollenberg’s specimen). The filaments were relatively long, from about 300 μm to more than 500 μm in length and consisting of more than 40 cells (Figs 15, 16). Cells of the erect filaments were 8–18 μm long and 3–14 μm broad, with a length to diameter ratio of 0.8:1 to 4.2:1. Cells at the distal end were more globose, while those at the lower end were elongated. Measurements of cell size may not be accurate due to the fairly deteriorated state of the specimen. Although an annotation on the syntype material indicated the presence of both unangia and plurangia, we did not observe unangia. The plurangia were more elaborate than those of species of *Mesospora* and were as illustrated by Saunders (1899; Plate 1, Fig. 4), measuring 9–14 μm in diameter with varying lengths of 80–150 μm, and capped by one to four sterile terminal cells (Figs 17, 18).

**HAPALOSPONDION SAXIGENUM** (Figs 19–21): The thallus was smooth, olive-green, mucilaginous upon contact with water and firmly adherent to the substratum without rhizoids (Fig. 19). The thallus consisted of long erect filaments (400–600 μm), which arose from a basal plate several cells thick. The erect filaments were simple, sometimes branched near the basal layer, loosely adherent and held together by mucilaginous material and comprised of approximately 40–60 cells. Cells of erect filaments were 3.7–8.1 μm wide and 6.3–12.9 μm long, with a length to diameter ratio of 0.9:1 to 3.2:1. Lower cells of the erect filaments were cylindrical, while the upper cells were more rounded. Unangia were inserted terminally on long stalks (approximately 20 to 35 cells) on which the cells were larger in dimension than those of the erect filaments (probably) arising directly from the basal plate, lateral to the middle of (or sub-superficial to) the surrounding filaments (Figs 20, 21). The unangia were elliptical, measuring 9.2–31.2 μm broad and 44.8–177.6 μm long. The stalk cells were 6.8–9.4 μm in diameter and 5.4–17.2 μm long, with a length to diameter ratio of 0.7:1 to 2.5:1. Plurangia were not observed.

**DISCUSSION**

The two-gene phylogenetic analyses revealed two new species of *Mesospora* that are genetically distinct from other species in the genus. Of the six currently recognised *Mesospora* species, four were included in the molecular analyses of this study and were shown to be genetically distinct from the two new species. Both *Mesospora indopacifica* and *Mesospora lombokensis* consistently formed independent and statistically well-supported clades in each analysis (Figs 1, 2). The newly described species were morpho-anatomically distinguished from one another as well as from other species of *Mesospora* based on a combination of characters (Table S2).

*Mesospora indopacifica* differs from *Mesospora schmidtii* by having more laterally cohesive erect filaments (especially near the basal portion) and unangia borne near the middle of the erect filaments vs erect filaments mutually free in their
entire length and unangia borne near the base of erect filaments in *M. schmidtii*. It is distinguished from *Mesospora elongata* by its shorter erect filaments (less than 20 cells per filament), shorter unangular stalks (one or two-celled) and more laterally cohesive erect filaments (especially near the basal portion) vs up to 30 cells per erect filament, long unangular stalks (up to 10 cells per stalk) and erect filaments mutually free in their entire length in *M. elongata*.

*Mesospora indopacifica* is distinguished from *Mesospora negrosensis* by consistently having two or three sterile cells capping plurangia. It is difficult to distinguish the species when the thalli are unangular, necessitating the use of

---

Fig. 13. *Mesospora pangoensis*. Two unangia borne terminally on short stalks (one- or two-celled) inserted lateral to loosely adherent erect filaments arising from a thin horizontal basal plate (voucher number: UC221298, holotype). Scale bar = 50 μm.

Fig. 14. *Basispora africana*. Relatively long and laterally free vegetative filaments arising from a prostrate system (voucher number: BM000770078). Scale bar = 100 μm.

Figs 15–18 *Hapalospongidion gelatinosum*.

Fig. 15. Assurgent filaments bearing plurangia, detached from the basal plate (voucher number: BM000563262; syntype) Scale bar = 100 μm.

Fig. 16. Relatively long and laterally free erect filaments bearing plurangia (voucher number: 39-80). Scale bar = 100 μm.

Fig. 17. Plurangia detached from erect filaments under higher (>40) magnification (voucher number: BM000563262; syntype). Scale bar = 50 μm.

Fig. 18. Dividing plurangia capped by sterile terminal cells (voucher number: 39-80). Scale bar = 50 μm.
molecular data. According to the literature, \textit{M. indopacifica} is distinguished from \textit{Mesospora macrocarpa} by the organisation of erect filaments (laterally adherent in the lower half portion vs loosely adherent in almost the entire length), the number of sterile terminal cells (two or three vs one) and the number of unangial stalk cells (one or two vs one or none). It differs from \textit{Mesospora vanbosseae} Børgesen by having unangia, a thin basal plate (two or three layers vs up to 10 or more layers), the organisation of erect filaments (laterally adherent in the lower half portion vs mutually free in the whole length) and the number of cells per erect filament (less than 20 vs 20 to 30).

\textit{Mesospora lombokensis} differs from \textit{Mesospora schmidtii} by the organisation of erect filaments (laterally cohesive in the lower half vegetative segment vs laterally free throughout), position of unangia (borne near the middle of the erect filaments vs borne near the base of the erect filaments) and the number of sterile terminal cells (one vs two). This new species is distinguished from \textit{Mesospora elongata} by the number of cells per erect filament (less than 20 vs up to 30), number of unangular stalk cells (one to three vs up to 10) and the organisation of erect filaments (laterally cohesive in the lower half vegetative segment vs laterally free throughout). Plurangia-bearing thalli of \textit{M. lombokensis} resemble \textit{Mesospora negrosensis} but the two can be distinguished by the smaller loculi of \textit{M. lombokensis}. Unangia-bearing thalli of both species are anatomically similar. The firmly adherent vegetative filaments in \textit{M. lombokensis} distinguish it from the loosely adherent erect filaments, which are easily separated by pressure in \textit{Mesospora macrocarpa}. The presence of unangia, a thin basal layer and laterally cohesive vegetative filaments differentiate \textit{M. lombokensis} from \textit{Mesospora vanbosseae}.

The type specimen (if any) of \textit{Mesospora vanbosseae} was not specified in the original publication (Børgesen 1924). This species was described based on specimens collected by C. Skottsberg from Hanga Piko, with plurangia but not unangia. Although both types of reproductive structures were reported for \textit{Mesospora macrocarpa}, details about its type specimen were not provided in the protologue (Feldmann 1931, 1935). Molecular analyses for \textit{M. vanbosseae} and \textit{M. macrocarpa} are lacking due to difficulties in collecting new specimens from type localities and in accessing voucher specimens. Consequently, the taxonomic status of \textit{M. vanbosseae} and \textit{M. macrocarpa} is yet to be determined. Despite their genetic variation with other species of \textit{Mesospora}, our current collection of \textit{Mesospora} sp. C and \textit{Mesospora} sp. D (most of them without reproductive structures) are insufficient for a formal description of the two undescribed species. Comparison of the morpho-anatomical characters was based on data from the literature (Table S2).

Sequence data from the holotype specimen of \textit{Mesospora pangoensis} suggested a close relationship with the generitype, \textit{Mesospora schmidtii}. This was further supported by morphological observations of the holotype. Setchell (1924) in

\textbf{Fig. 19.} Thallus encrusting on substratum. Scale bar = 5 mm.
\textbf{Fig. 20.} Single unangium (indicated by arrow) borne terminally on a long stalk inserted among laterally free erect filaments (under ×10 magnification). Scale bar = 200 μm.
\textbf{Fig. 21.} Single unangium borne terminally on a long stalk inserted among laterally free erect filaments (under ×20 magnification). Scale bar = 100 μm.
his description of *Ralfsia pangoensis* (transferred to *Mesospora* by Tanaka & Chihara in 1982) recognised the similarities between *M. pangoensis* and *M. schmidtii* but stated that they can be differentiated by the shape of the erect filaments and the position of unangia. Setchell (1924) probably misinterpreted Weber–van Bosse’s (1913) description of the unangia of *M. schmidtii*, which are, in fact, inserted terminally on a few-celled stalk borne laterally from the base of the surrounding erect filaments (compare Fig. 13 of this study with Fig. 3d of Poong et al. 2014). Our examination of the holotype of *M. pangoensis* indicates that this species is similar to *M. schmidtii* on the basis of the number of cells per erect filament, thickness of the basal layer, position of the unangia and the number of unangial stalk cells. We consider *M. pangoensis* and *M. schmidtii* as distinct (albeit cryptic) species, at least until additional specimens can be examined.

The shape, size and colour of the thallus and the size of unangia are highly variable within each species and are subject to environmental conditions and age of the individual (personal observations of S.-W. Poong, data not shown). For instance, the diameter of an individual thallus is always difficult to determine because several thalli can be confluent. Taxonomically reliable characters, consistent within a species regardless of environmental conditions and age, include the number of sterile cells terminating the plurangia, the number of unangial stalk cells, the position of unangia and the organisation of the erect filaments.

In addition to discovering two new species of *Mesospora*, this study presents a new record of *Mesospora elongata* for Malaysia. The most commonly reported species of *Mesospora* is *Mesospora schmidtii*, found mainly in the Pacific Ocean (Indo-Malay, Poong et al. 2013; Australia, Bostock & Holland 2010; Solomon Islands, Womersley & Bailey 1970) and the Indian Ocean (Krishnamurthy & Baluswami 1986). *Mesospora schmidtii* was not included in a molecular phylogenetics study on crustose brown algae from Japan by Lim et al. (2007), who reported three undescribed species of *Mesospora*. Based on the descriptions and illustrations by Kaehler (1994), it is likely that the record of *M. schmidtii* in Hong Kong SAR actually represents another species. The distribution of the generitype might therefore be restricted to the tropical coasts of the Indo-Pacific but sampling with a larger geographical coverage is necessary to confirm this.

The distributional ranges of *Mesospora negrosensis* (Malaysia and the Philippines) and *Mesospora elongata* (Japan, Indonesia and Malaysia) are currently limited to the Indo-Pacific region (Poong et al. 2014). *Mesospora vanhossea*, which was first reported from Easter Island, is recorded only in the Pacific (Santelices & Abbott 1987). *Mesospora macrocarpa* appears to be confined to the Mediterranean Sea. At present, *Mesospora lombokensis* is limited to Lombok Island, Indonesia, while *Mesospora indopacifica* is reported from eastern Australia, the east coast of Peninsular Malaysia and Lombok Island, Indonesia. To obtain a better understanding of the geographic distribution of species within *Mesospora*, a comprehensive sampling at a global scale is necessary.

Since the establishment of *Hapalospondion* by Saunders (1899) based on *Hapalospondion gelatinosum* from North America, Lindauer (1949) added two new species from New Zealand, *Hapalospondion saxigenum* and *Hapalospondion durvilleae* Lindauer, to the genus. Womersley (1987) added *Hapalospondion capitatum* Womersley from Australia. More recently another species, *Hapalospondion thirumullavaranmense* Sophiammal Nettar & Panikkar was reported from Kerala, India (Sophiammal Nettar & Panikkar 2009). Thus far, unangia have not been reported for *H. capitatum* and *H. thirumullavaranmense* (Table S3). Lindauer (1949) distinguished *H. saxigenum* from the generitype because it lacked plurangia and had a poorly developed basal layer. Womersley (1987) distinguished *H. capitatum* and *H. thirumullavaranmense* based on the former’s erect filaments with larger, capitate, uppermost two to three cells and more elaborately plurangia; and from *H. saxigenum*, which lacks the capitate erect filaments and is known only with unangia. Sophiammal Nettar & Panikkar (2009) did not distinguish *H. thirumullavaranmense* from other species of *Hapalospondion* but they mentioned that the species differed from *Mesospora schmidtii* in the number of cells of the basal disc, size of the apical cells and presence of lateral collar-like extensions. The type specimens of *H. capitatum* and *H. thirumullavaranmense* were requested but were not available for examination.

*Hapalospondion* and *Mesospora* can be distinguished based on the number of cells in the erect filaments. Erect filaments of *Mesospora* are considerably shorter (generally less than 20 cells) as acknowledged by Womersley (1987) compared with *Hapalospondion*, in which erect filaments generally comprised more than 40 cells (up to 60 cells in both *Hapalospondion saxigenum* and *Hapalospondion capitatum*; up to 85 cells in *Hapalospondion thirumullavaranmense*). In *Hapalospondion*, unangia are inserted terminally on long stalks (described by Saunders as shorter erect filaments) arising (directly? as illustrated in Plate 1, Fig. 2 of Saunders [1899]) from the basal layer. Saunders (1899) did not mention the number of unangial stalk cells for *Hapalospondion gelatinosum*, while Lindauer (1949) mentioned that the unangial stalks for *H. saxigenum* were of 12 cells or more. In contrast, the unangia in *Mesospora* are inserted terminally on shorter stalks of less than 12 cells, issuing laterally from erect filaments. In this study, the unangial stalks of *H. saxigenum* consisted of 20 to 35 cells.

John & Lawson (1974) transferred *Hapalospondion saxigenum* and *Hapalospondion durvilleae* to *Basisporia*, and the latter was separated from the other two species of *Basisporia* due to its parasitic nature. In the same year, South (1974) erected the genus *Herpodiscus* with *Herpodiscus durvilleae* (Lindauer) South as the type, and the alga was referred to Elachistaceae of the Chordariales. A molecular study by Heesch et al. (2008) has shown that this parasitic taxon is affiliated with the Sphacelariales.

*Basisporia africana*, the generitype, is described as having a thallus consisting of three to several layers of prostrate filaments bearing assimilant filaments, free and closely packed, consisting of up to 40 cells, distinctly clavate toward the upper end with several discoid chloroplasts per cell; with plurangia being unknown. *Basisporia*, like *Hapalospondion* and *Mesospora*, has erect filaments loosely attached to one another by mucilage and readily separated on squashing. Womersley (1987) thought that *Mesospora* and *Hapalospondion* might not be distinct. He pointed out that in both,
there are one to three chloroplasts per cell (the number depending on the size and age of the cells) and unangia terminal on vegetative filaments that are facultatively basally branched. He further noted that Basispora is similar to Hapalospongidion in possessing several chloroplasts per cell and unangia arising terminally on long stalks from near the base of the laterally free erect filaments. Following Womersley (1987), Silva et al. (1996) transferred Mesospora schmidtii to Hapalospongidion and later León-Alvarez & González-González (1993) did the same for Mesospora macrocarpa and Mesospora vanbosseae.

John & Lawson (1974) and Tanaka & Chihara (1982) pointed out the close relationship between the three genera, which share the following characters: crustose plants with a base of prostrate filaments from which simple, loosely adjoined filaments arose; intercalary plurangia and unangia originating terminally on stalks. Like Mesospora, Basispora may be distinguished from Hapalospongidion based on the number of cells in the erect filament and the number of unangial stalk cells. The type species of Basispora is reported to have vegetative filaments of up to 40 cells and stalks consisting of (4) 6–10 (15) cells.

John & Lawson (1974) considered Hapalospongidion saxigenum to be a member of Basispora, with multiple chloroplasts per cell and unangia borne on distinct stalks arising from near the base of the erect filaments. Buchanan (2005) considered the multiple plastids observed by Lindauer (1949) to be a misidentification of physodes, since the New Zealand specimens he examined possessed only a single plastid in each cell. Rull Lluch (2002) commented that Basispora should be kept separate until the number and morphology of chloroplasts are clarified. According to Rull Lluch (2002), Hapalospongidion is characterised by the presence of one to several blade-like chloroplasts rather than several discoid chloroplasts as originally ascribed to Basispora by John & Lawson (1974). The observational inconsistency is noteworthy.

John & Lawson (1974) and Tanaka & Chihara (1982) described the unangial stalks in Hapalospongidion as indistinguishable from ordinary vegetative erect filaments but a careful review of the description of Saunders (1899) of longer and shorter erect filaments highlights the difference in cell dimensions between the two types of filaments. Unangia were absent in the Hapalospongidion gelatinosum syntype and Hollenberg’s specimen examined in this study. Morphological observations of Hapalospongidion saxigenum (Figs 19–21) showed stalks morphologically differentiated from the vegetative erect filaments. However, it is unclear whether these stalks originate laterally from the base of erect filaments or were inserted directly on the basal plate. Hollenberg (1942) retained Mesospora as a distinct genus despite the similarities between this genus and Hapalospongidion.

Given the above considerations, we consider that the merger of Mesospora and Basispora with Hapalospongidion as proposed by Womersley (1987) and adopted by León-Alvarez & González-González (1993), Silva et al. (1996) and Parente et al. (2006) is premature, and the appropriate taxonomic conclusions awaited detailed molecular studies to resolve the phylogenetic relationships between these taxa. The molecular analysis in this study (Fig. 1), although not conclusive until more authentic specimens of Hapalospongidion and Basispora are included, does not indicate support for the merger of the three genera. The fact that the cox1 gene could not be amplified for the Hapalospongidion and Basispora specimens suggests that the primer annealing sites differ from those in species of Mesospora or that a very large intron interferes with amplification. It is evident that further investigation is necessary to determine the taxonomic status of the three genera considered here, due to the inconsistencies of characters observed by different authors. This study provides a basis for the reinstatement of the genus Mesospora that can be clearly distinguished from Hapalospongidion on morpho-anatomical and molecular grounds.

ACKNOWLEDGEMENTS

This work is funded by University of Malaya-High Impact (UM.C/625/1/HIR,088) and Ministry of Higher Education-High Impact Research (H-50001-00-A000025) grants to P.-E. Lim. We thank the referees whose constructive and valuable comments improved the manuscript. We appreciate the assistance by Jo Wilbrham from the Natural History Museum for the loans of H. gelatinosum and B. africana.

SUPPLEMENTARY DATA

Supplementary data associated with this article can be found online at http://dx.doi.org/10.2216/16-14.1.s1.

REFERENCES


