Mycobiology

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/tmyb20

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To cite this article: Wonjun Lee, Dong-Geon Kim, Rekhani H. Perera, Ji Seon Kim, Yoonhee Cho, Jun Won Lee, Chang Wan Seo & Young Woon Lim (27 Nov 2023): Diversity of *Nigrospora* (*Xylariales*, *Apiosporaceae*) Species Identified in Korean Macroalgae Including Five Unrecorded Species, Mycobiology, DOI: 10.1080/12298093.2023.2283272

To link to this article: https://doi.org/10.1080/12298093.2023.2283272

MYCOBIOLOGY
https://doi.org/10.1080/12298093.2023.2283272

RESEARCH ARTICLE

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Diversity of *Nigrospora* (*Xylariales*, *Apiosporaceae*) Species Identified in Korean Macroalgae Including Five Unrecorded Species

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ABSTRACT

Nigrospora (*Xylariales*, *Apiosporaceae*) consists of species of terrestrial plant endophytes and pathogens. *Nigrospora* has also been reported in marine environments such as mangroves, sea fans, and macroalgae. However, limited research has been conducted on *Nigrospora* associated with macroalgae. Here, we isolated *Nigrospora* species from three types of algae (brown, green, and red algae) from Korean islands (Chuja, Jeju, and Ulleung) based on phylogenetic analyses of multigenetic markers: the internal transcribed spacers (ITS), beta-tubulin (*BenA*), and translation elongation factor 1 (*TEF1-α*). A total of 17 *Nigrospora* strains were isolated from macroalgae and identified as nine distinct species. The majority of *Nigrospora* species (seven) were found on brown algae, followed by red algae (three), and then green algae (two). To our understanding, this study represents the first account of *N. cooperae*, *N. covidalis*, *N. guilinensis*, *N. lacticolonia*, *N. osmanthi*, *N. pyriformis*, and *N. rubi* occurring in marine environments. Additionally, this study provides the first report of the occurrence of *N. cooperae*, *N. covidalis*, *N. guilinensis*, *N. lacticolonia*, and *N. osmanthi* in South Korea. This study will provide valuable insights for future research exploring the functions of fungi in macroalgal communities.

ARTICLE HISTORY
Received 19 July 2023
Revised 6 November 2023
Accepted 9 November 2023

KEYWORDS
Marine fungi; taxonomy; seaweed; algicolous fungi; *Ascomycota*

1. Introduction

Nigrospora Zimm. is characterized by large dark conidiospores [1]. Since the first report of the type species, *N. panici*, from leaves in Indonesia [1], *Nigrospora* species have been reported globally [2–6]. The phylogenetic analysis based on the internal transcribed spacer (ITS), beta-tubulin (*BenA*), and translation elongation factor 1-a (*TEF1-α*) affirmed the placement of *Nigrospora* in *Apiosporaceae* of *Xylariales* [7]. Up to date, 44 *Nigrospora* species have been recorded in the MycoBank database (https://www.mycobank.org/; accessed on 2023.06.14). Among them, 33 species have DNA sequence data in GenBank.

Nigrospora is usually reported to occur in terrestrial environments such as indoors [8,9], lichens [5,10], and plants [4,11], but it has also been reported to occur in marine environments. Specifically, *Nigrospora oryzae* and *Nigrospora sphaerica* have predominantly been isolated from marine organisms such as corals [12], mangroves [13–15], macroalgae [16–19], sea fans [19,20], and sponges [21,22]. Furthermore, *Nigrospora camelliae-sinensis* has been isolated from a mangrove [23], and *Nigrospora aurantiaca* has been found in sponges [24]. Most of these studies aimed at discovering bioactive compounds derived from *Nigrospora* rather than exploring its diversity or ecological interactions.

Macroalgae are integral components of marine ecosystems, providing habitats for diverse organisms and contributing to carbon sequestration [25–27]. Microbial associations with macroalgae have been extensively studied [28,29]. Bacterial communities associated with macroalgae have been found to play roles in nutrient supply to macroalgae, defense against unwanted colonization, and even morphogenesis of macroalgae [28]. However, fungal contributions to macroalgae remain poorly understood. Given the significant value of macroalgae and their microorganisms, it is crucial to investigate the relationship between macroalgae and fungi to gain a comprehensive understanding of their ecological significance and potential application.

In this study, we investigated i) *Nigrospora* species inhabiting macroalgae collected from three Korean islands (Chuja, Jeju, and Ulleung) and ii) whether they have a specific correlation with algal types. We isolated 17 *Nigrospora* strains from brown, green, and red algae. Nine *Nigrospora*

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species were identified at the species level based on both multigenetic markers (ITS, *BenA*, and *TEF1-α*) and morphological analysis. Seven of these species are, for the first time, reported to be associated with macroalgae and marine environments. Brown algae exhibited the highest level of *Nigrospora* species diversity of the three algal types.

2. Materials and methods

2.1. Sampling and fungal isolation

Fourteen macroalgae samples were collected from three islands (Chuja, Jeju, and Ulleung) in South Korea in August of 2018 and 2021 (Table 1). Macroalgae were morphologically identified according to [30] and Marine Bio-Resource Information System (https://www.mbris.kr/pub/info/encyclopedia/algae.do; accessed on 2023.05.17).

Macroalgae samples were cut into $0.5 \times 0.5\,cm^2$ pieces and placed on dichloran rose bengal chloramphenicol (DRBC) agar (Difco, Sparks, MD, USA) media supplemented with sterilized seawater (SSW). Fungal colonies grown from the samples were isolated and transferred to potato dextrose agar (PDA; Difco, Sparks, MD, USA) media supplemented with SSW. The living cultures of each isolate were stocked in 20% (v/v) glycerol at −80 °C and deposited into the Seoul National University Fungus Collection (SFC).

2.2. Molecular analyses (DNA extraction, PCR amplification, sequencing, and phylogenetic analysis)

The mycelium of each fungal isolate grown on PDA was ground by a Bead Ruptor Elite Homogenizer (OMNI International, Kennesaw, GA, USA). DNA extraction was conducted using an AccuPrep® Genomic DNA Extraction Kit (Bioneer, Daejeon, South Korea) following the manufacturer's protocol with a small modification where cetyltrimethylammonium bromide (CTAB) extraction solution (Biosesang, Incheon, South Korea) was used instead of the TL buffer included in the kit.

The ITS region was amplified by PCR using a C1000 thermal cycler (Bio-Rad, Richmond, CA, USA) with the primer sets ITS1F/ITS4 [31,32]. *BenA* and *TEF1-α* were subsequently amplified with Bt2a/Bt2b [33] and EF1-728F/EF2 [34,35] primers, respectively. The PCR conditions were as follows: initial denaturation at 95 °C for 5 min, 35 cycles of denaturation at 95 °C for 40 s, annealing at 55 °C for 40 s, and extension at 72 °C for 60 s, followed by a final extension at 72 °C for 5 min. Purification was done using an Expin™ PCR SV kit (GeneAll Biotechnology, Seoul, South Korea), following the manufacturer's protocol. Sanger sequencing was performed in both forward and reverse directions using the PCR primers in an ABI prism 3730xl Genetic Analyzer (Life Technologies, Gaithersburg, MD, USA) at Macrogen (Seoul, South Korea). Obtained sequences were merged using the *De novo assemble* function in the Geneious Prime software ver. 2023. 1. 1. (Biomatters Ltd., San Diego, CA, USA) and were then proofread and edited manually. The proofread sequences were deposited in GenBank (Table 1).

The generated sequences, reference GenBank *Nigrospora* sequences, and an outgroup sequence of *Apiospora sargassi* (KUC 21287) were aligned by each genetic marker (ITS, *BenA*, and *TEF1-α*) using MAFFT v7.490 [36] in the Geneious Prime software ver. 2023.1.1. (Biomatters Ltd., San Diego, CA, USA). The alignments were then concatenated. The best model test was investigated in MEGA 7.0.26 [37] to conduct the maximum likelihood analysis. The phylogenetic tree was inferred through RAxML analysis [38] with 1,000 replications using the GTR GAMMA model in the Geneious Prime software.

2.3. Morphological observation

For an effective observation and measurement of microscopic features, *Nigrospora* strains were initially subcultured on PDA and subsequently transferred to both PDA and synthetic nutrient-poor agar media (SNA; KH_2PO_4 1 g, KNO_3 1 g, $MgSO_4{\cdot}7H_2O$ 5 g, KCl 0.5 g, Glucose 0.2 g, Saccharose 0.2 g, and Bacto agar 20 g per 1 L). The colonies on PDA were incubated for 7 d at 25 °C in the dark to observe the culture morphology. The color of the colonies was determined using the Methuen Handbook of Color [39]. Representative strains of each species were cultivated on SNA for conidial structure observation. All observations were done using a Nikon 80i light microscope (Tokyo, Japan). At least 30 measurements were obtained per strain to calculate the mean size of the microscopic structures. The colonies and the conidial structures were measured using ImageJ software [40].

3. Results

Seventeen *Nigrospora* strains were isolated from macroalgae, and ITS and two protein-coding genes (*BenA* and *TEF1-α*) were used to infer a maximum likelihood tree to identify the strains at the species level. The total number of molecular characters was 1,853 (586 in ITS, 402 in *BenA*, and 865 in *TEF1-α*). Nine *Nigrospora* species (*N. aurantiaca*, *N. cooperae*, *N. covidalis*, *N. guilinensis*, *N. lacticolonia*, *N. oryzae*, *N. osmanthi*, *N. pyriformis*, and *N. rubi*) were identified through the phylogenetic analysis (Figure 1).

Table 1. List of strains, collection information, and GenBank accession numbers of sequences used in the phylogenetic analysis. Newly reported strains are indicated in bold and holotypes are indicated by "*".

Species	Strain number	Habitat/host	Country	GenBank accession numbers		
				ITS	*BenA*	*TEF1-α*
Apiospora sargassi	KUC21287	*Sargassum fulvellum*	Jeju, South Korea	MF615227	MF615232	MN868934
Nigrospora aurantiaca	CGMCC 3.18130* = LC 7302	*Nelumbo* sp.	China	KX986064	KY019465	KY019295
	SFC20230324-M01	***Polyopes* sp. (*Rhodophyta*)**	**Chuja, South Korea**	**OQ726356**	**OQ735177**	**OQ735194**
	SFC20230324-M02	***Hypnea* sp. (*Rhodophyta*)**	**Chuja, South Korea**	**OQ726355**	**OQ735178**	**OQ735195**
N. bambusae	CGMCC 3.18327* = LC 7114	Bamboo (leaf)	China	KY385307	KY385319	KY385313
N. cooperae	BRIP 72440a*	*Heteropogon contortus*	Australia	OP035048	OP039540	OP039539
	SFC20230324-M03	***Ishige* sp. (*Phaeophyceae*)**	**Chuja, South Korea**	**OQ726361**	**OQ735179**	**OQ735196**
N. camelliae-sinensis	CGMCC 3.18125* = LC 3500	*Camellia sinensis*	China	KX985986	KY019460	KY019293
N. covidalis	CGMCC 3.20538*	*Lithocarpus* sp.	China	OK335209	OK431479	OK431485
	SFC20230324-M04	***Ishige* sp. (*Phaeophyceae*)**	**Chuja, South Korea**	**OQ726371**	**OQ735180**	**OQ735197**
N. chinensis	CGMCC 3.18127* = LC 4575	*Machilus breviflora*	China	KX986023	KY019462	KY019422
N. falsivesicularis	CGMCC 3.19678*	*Saccharum officinarum*	China	MN215778	MN329942	MN264017
N. globosa	CGMCC 3.19633*	Soil of cave	China	MK329121	MK336134	–
N. globospora	CGMCC 3.20539*	*Petasites hybridus*	China	OK335211	OK431481	OK431487
N. gorlenkoana	CBS 480.73*	*Vitis vinifera*	Kazakhstan	KX986048	KY019456	KY019420
N. guangdongensis	CFCC 53917	*Cunninghamia lanceolata* (needle)	China	MT017509	MT024495	MT024493
N. guilinensis	CGMCC 3.18124* = LC 3481	*Camellia sinensis*	China	KX985983	KY019459	KY019292
	SFC20230324-M05	***Sargassum* sp. (*Phaeophyceae*)**	**Ulleung, South Korea**	**OQ726362**	**OQ735181**	**OQ735198**
N. hainanensis	CGMCC 3.18129* = LC 7030	*Musa paradisiaca* (leaf)	China	KX986091	KY019464	KY019415
N. lacticolonia	CGMCC 3.18123* = LC 3324	*Camellia sinensis*	China	KX985978	KY019458	KY019291
	SFC20230324-M06	***Sargassum* sp. (*Phaeophyceae*)**	**Chuja, South Korea**	**OQ726357**	**OQ735182**	**OQ735199**
N. macarangae	MFLUCC 19-0141*	*Macaranga tanarius*	Taiwan	MW114318	–	–
	NCYUCC 19-0177	*Macaranga tanarius*	Taiwan	MW114319	–	–
N. magnoliae	MFLUCC 19-0112*	*Magnolia liliifera*	China	MW285092	MW438334	–
N. musae	CBS 319.34*	*Musa paradisiaca* (fruit)	Australia	KX986076	KY019455	KY019419
N. oryzae	LC 7306	*Nelumbo* sp. (leaf)	China	KX986068	KY019612	KY019408
	LC 2689	*Rhododendron* sp.	China	KX985942	KY019469	KY019423
	LC 4265	*Rhododendron* sp.	China	KX985994	KY019518	KY019335
	LC 4338	*Camellia* sp.	China	KX986008	KY019532	KY019349
	SFC20230324-M07	***Ulva* sp. (*Chlorophyta*)**	**Jeju, South Korea**	**OQ726369**	**OQ735183**	**OQ735200**
	SFC20230324-M08	***Myagropsis* sp. (*Phaeophyceae*)**	**Ulleung, South Korea**	**OQ726367**	**OQ735184**	**OQ735201**
	SFC20230324-M09	***Chondria* sp. (*Rhodophyta*)**	**Ulleung, South Korea**	**OQ726368**	**OQ735185**	**OQ735202**
	SFC20230324-M10	***Grateloupia* sp. (*Rhodophyta*)**	**Chuja, South Korea**	**OQ726366**	**OQ735186**	**OQ735203**
N. osmanthi	CGMCC 3.18126* = LC 4350	*Osmanthus* sp.	China	KX986010	KY019461	KY019421
	SFC20230324-M11	***Codium* sp. (*Chlorophyta*)**	**Jeju, South Korea**	**OQ726360**	**OQ735187**	**OQ735204**
	SFC20230324-M12	***Ulva* sp. (*Chlorophyta*)**	**Jeju, South Korea**	**OQ726358**	**OQ735188**	**OQ735205**
	SFC20230324-M13	***Codium* sp. (*Chlorophyta*)**	**Jeju, South Korea**	**OQ726359**	**OQ735189**	**OQ735206**
N. philosophiae-doctoris	CGMCC 3.20540*	*Disporum sessile*	China	OK335213	OK431483	OK431489
N. pyriformis	CGMCC 3.18122* = LC 2045	*Citrus sinensis*	China	KX985940	KY019457	KY019290
	SFC20230324-M14	***Sargassum* sp. (*Phaeophyceae*)**	**Chuja, South Korea**	**OQ726363**	**OQ735190**	**OQ735207**
	SFC20230324-M15	***Polyopes* sp. (*Rhodophyta*)**	**Chuja, South Korea**	**OQ726364**	**OQ735191**	**OQ735208**
	SFC20230324-M16	***Laurencia* sp. (*Rhodophyta*)**	**Chuja, South Korea**	**OQ726365**	**OQ735192**	**OQ735209**
N. rubi	CGMCC 3.18326* = LC 2698	*Rubus* sp.	China	KX985948	KY019475	KY019302
	SFC20230324-M17	***Sargassum* sp. (*Phaeophyceae*)**	**Chuja, South Korea**	**OQ726370**	**OQ735193**	**OQ735210**
N. saccharicola	CGMCC 3.19362*	*Saccharum officinarum*	China	MN215788	MN329951	MN264027
N. sacchari-officinarum	CGMCC 3.19335*	*Saccharum officinarum*	China	MN215791	MN329954	MN264030
N. singularis	CGMCC 3.19334*	*Saccharum officinarum*	China	MN215793	MN329956	MN264032
N. sphaerica	LC 2840	*Harpullia longipetala*	China	KX985965	KY019492	KY019318
	LC 2958	*Cleyera japonica*	China	KX985966	KY019493	KY019319
	LC 4372	*Rhododendron arboreum*	China	KX986012	KY019535	KY019351
	LC 6969	*Musa paradisiaca* (leaf)	China	KX986077	KY019584	KY019386
N. vesicularifera	CGMCC 3.19333*	*Saccharum officinarum*	China	MN215812	MN329975	MN264051
N. vesicularis	CGMCC 3.18128* = LC 7010	*Musa paradisiaca* (leaf)	China	KX986088	KY019463	KY019294
N. zimmermanii	CBS 290.62*	*Saccharum officinarum* (leaf)	Ecuador	KY385309	KY385317	KY385311

Each strain matched its corresponding species with at least 98% bootstrap support.

Nigrospora species were categorized based on the algal types to which they were associated (Figure 1). The macroalgae were morphologically identified as brown (*Sargassum* spp., *Ishige okamurae*, and *Myagropsis myagroides*), green (*Ulva* sp. and *Codium fragile*), and red algae (*Hypnea* sp., *Polyopes* sp., *Laurencia* sp., *Grateloupia* sp., and *Chondria* sp.) (Table 1). The largest number of *Nigrospora* species (seven spp.)—*N. cooperae*, *N. covidalis*, *N. guilinensis*, *N. lacticolonia*, *N. oryzae*, *N. pyriformis*, and *N. rubi*—were isolated from brown algae. Three *Nigrospora* species (*N. aurantiaca*, *N. oryzae*, and *N. pyriformis*) were isolated from red algae. *Nigrospora oryzae* and *N. osmanthi* were isolated from green algae. *Nigrospora oryzae* appeared on all types of algae, and *N. pyriformis* was detected in both brown and red algae (Figure 1).

4. Taxonomy

Nigrospora cooperae Y.P. Tan, Bishop-Hurley, Bransgr. & R.G. Shivas (2022) (Figure 2A).

Sexual morph: Undetermined. Asexual morph on SNA: *Hyphae* branched, guttulate, septate, pale brown, 1.6–5.4 µm diam. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* monoblastic, discrete,

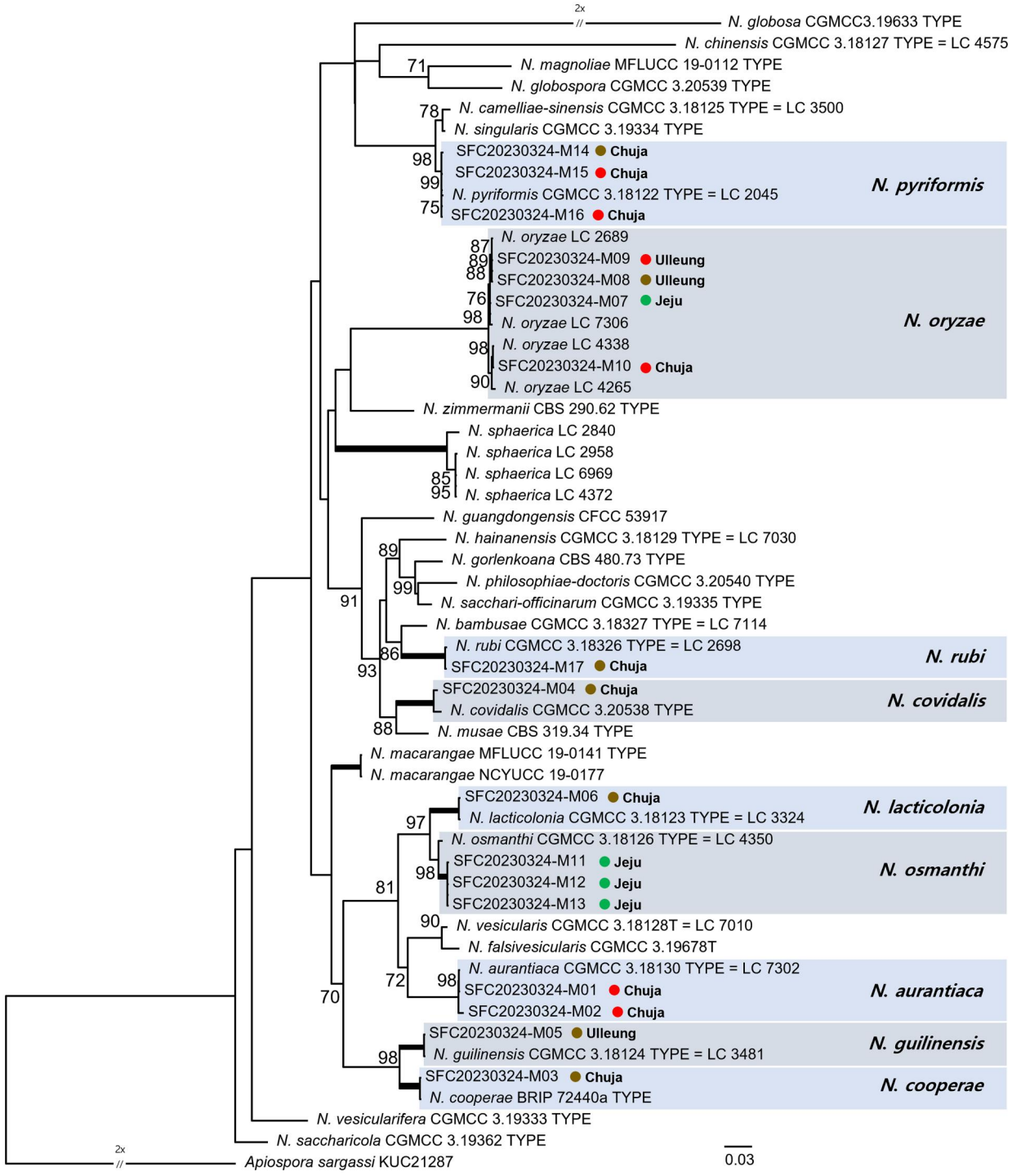

Figure 1. The maximum likelihood tree of *Nigrospora* species with outgroup *Apiospora sargassi* (KUC21287). ITS, *BenA*, and *TEF1-α* genetic markers were used in phylogenetic analyses. The newly collected strains are enclosed in colored boxes. Bootstrap values of more than 70% are shown at the nodes. Branches that lead to nodes of bootstrap values of 100 are indicated by a bold line. Colored circles indicate the color of algae (brown, green, and red) where each strain was isolated, followed by the island on which the strain was collected.

pale brown, doliiform to ampulliform to subglobose, 5.6–13.9 × 4–7 µm (av. = 8.6 ± 2.07 × 5.47 ± 0.83). *Conidia* solitary, spherical or ellipsoidal, aseptate, black, shiny, smooth-walled, spherical 10.3–14 µm (av. = 12.2 ± 0.84), ellipsoidal 11.4–14.6 × 8.2–11.4 µm (av. = 12.67 ± 0.84 × 9.63 ± 0.79).

Culture characters on PDA: Colonies sparse, velvety, fimbriate, irregular at the margin, surface gray-green (1C4) to bile yellow (30C5), reverse concolorous, not producing pigments in PDA, with prominent exudates, reaching 18–45 mm diameter in 7 d at 25 °C.

Materials examined: South Korea. South Sea, Chuja island, 33°57′11″N, 126°18′07″E, from *Ishige* sp. (*Phaeophyceae*), 31 August 2021, M. S. Park & Y. W. Lim (SFC20230324-M03, stored in a metabolically inactive state).

Notes: SFC20230324-M03 produces prominent exudates, and the conidiogenous cells of this isolate

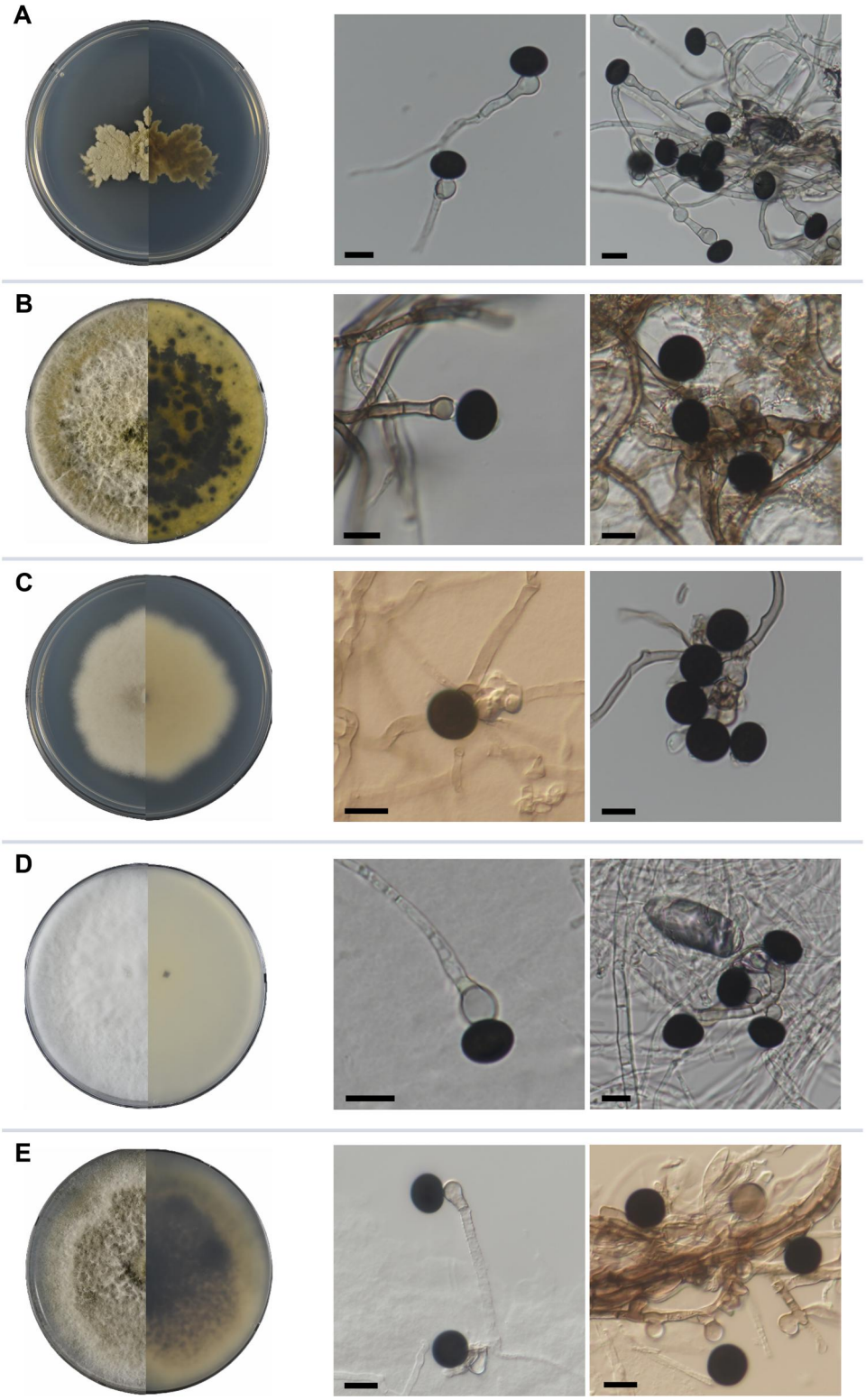

Figure 2. Cultures of five *Nigrospora* species from this study. On the left, surface (left) and reverse (right) sides of strains on PDA are shown in halves. On the right, conidial structures on SNA media are shown. (A) *N. cooperae*, (B) *N. covidalis*, (C) *N. guilinensis*, (D) *N. lacticolonia*, and (E) *N. osmanthi*. Scale bar: 10 µm.

displayed a range of length variations. However, the original description of *N. cooperae* does not produce any prominent exudates, and the length variation (7–10 µm) of conidiogenous cells in the holotype is lower than our isolate [41].

Nigrospora covidalis M. Raza, Qian Chen & L. Cai. (2017) (Figure 2B).

Sexual morph: Undetermined. Asexual morph on SNA: *Hyphae* branched, septate, guttulate, hyaline to pale brown, 1.8–4.6 µm diam. *Conidiophores* monoblastic, flexuous or straight, pale brown, and some conidiophores reduced to conidiogenous cells. *Conidiogenous cells* monoblastic, discrete, pale brown, doliiform to ampulliform, 5.4–12.7 × 3.7–8.8 µm (av. = 8.24 ± 1.81 × 6.32 ± 1.32). *Hyaline vesicles*

delimited the conidia from their conidiogenous cells. *Conidia* sparse, solitary, spherical or ellipsoidal, aseptate, mostly black, discrete on aerial hyphae, spherical 10.9–15.2 µm diam. (av. = 12.9 ± 1.21), ellipsoidal 11.8–15.8 × 8.8–12.3 µm (av. = 13.86 ± 1.12 × 11.03 ± 0.81).

Culture characters on PDA: Colonies floccose, surface white (1A1) to light grey (1D1), sometimes deep green (1C8), reverse pale grey (1B1) to grayish yellow (1B4) with black patches, mostly producing yellow pigments in PDA, reaching 90 mm diameter in 4–5 d at 25 °C.

Materials examined: South Korea. South Sea, Chuja islands, 33°57′11″N, 126°18′07″E, from *Ishige* sp. (*Phaeophyceae*), 31 August 2021, M. S. Park & Y. W. Lim (SFC20230324-M04, stored in a metabolically inactive state).

Notes: *Nigrospora covidalis* can be morphologically distinguished from *N. musae*, which is phylogenetically a sister species, by the smaller size of its conidia [7,41]. Even though the absence of vesicles is a taxonomic key to delimiting *N. covidalis* from *N. musae* [7], hyaline vesicles are observed in this isolate. Furthermore, SFC20230324-M04 produces yellow pigment and grew faster on PDA media compared to what is reported in the original description [42].

Nigrospora guilinensis Mei Wang & L. Cai (2017) (Figure 2C).

Sexual morph: Undetermined. Asexual morph on SNA: *Hyphae* branched, smooth, hyaline to pale brown, septate, 1.9–6.1 µm diam. *Conidiophores* usually reduced to conidiogenous cells, aggregated in clusters on hyphae. *Conidiogenous cells* monoblastic, determinate, hyaline to pale brown, smooth-walled, doliiform to ampulliform, in clusters on aerial mycelia, 5.0–13.6 × 3.7–12.9 µm (av. = 8.02 ± 2.12 × 6.53 ± 1.93). *Conidia* solitary, spherical or ellipsoidal, aseptate, black, shiny, smooth-walled, spherical, 10.8–13.4 µm diam. (av. = 12 ± 0.7), ellipsoidal, 11.4–14.2 × 8.7–11.2 µm (av. = 12.77 ± 0.66 × 9.97 ± 0.63).

Culture characters on PDA: Colonies wooly, cottony, margin irregular, undulate, surface and reverse white (1A1) with a few black patches, sometimes producing red pigment, reaching 54–68 mm diameter after 7 d at 25 °C.

Materials examined: South Korea. East Sea, Ulleung island, 37°30′52″N, 130°47′41″E, from *Sargassum* sp. (*Phaeophyceae*), 29 August 2018, M. S. Park & Y. W. Lim (SFC20230324-M05, stored in a metabolically inactive state).

Notes: *Nigrospora guilinensis* can be distinguished from closely related species by morphological characteristics such as the ability to produce diffusible pigment on PDA and the arrangement of conidiogenous cells [7]. Nevertheless, pigment production is not consistently observed in SFC20230324-M05, and the isolate forms wider conidiogenous cells (6–11 × 4–7.5 µm) compared to that reported in the original description [7].

Nigrospora lacticolonia Mei Wang & L. Cai. (2017) (Figure 2D).

Sexual morph: Undetermined. Asexual morph on SNA: *Hyphae* branched, smooth, hyaline, septate, 1.5–4.6 µm diam. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* sometimes aggregated in clusters on hyphae, pale brown, smooth-walled, mostly spherical, sometimes doliiform, 5–13.2 × 4–8 µm (av. = 8.63 ± 1.89 × 6.09 ± 1.02). *Conidia* sparse, solitary, spherical or ellipsoidal, aseptate, black, shiny, smooth-walled, spherical 9.7–15.1 µm diam. (av. = 12.28 ± 1.45), ellipsoidal 10.2–15.2 × 7.9–12.1 µm (av. = 12.52 ± 1.13 × 9.73 ± 1.03).

Culture characters on PDA: Colonies floccose, entire edge, surface, and reverse white (1A1), without any patches, reaching 90 mm diameter in 3–4 d at 25 °C.

Materials examined: South Korea. South Sea, Chuja island, 33°57′11″N, 126°18′07″E, from *Sargassum* sp. (*Phaeophyceae*), 31 August 2021, M. S. Park & Y. W. Lim (SFC20230324-M06, stored in a metabolically inactive state).

Notes: *Nigrospora lacticolonia* derived its name from the creamy white colonies on PDA [7]. Similarly, the isolate SFC20230324-M06, from *Sargassum* sp., colonized PDA in white. SFC20230324-M06 sparsely produces conidia and does not show a prominent tendency to aggregate conidiogenous cells in clusters. Moreover, narrower ellipsoidal conidia are observed than those in the original description (13.5–17.5 × 10.5–13.5 µm) [7].

Nigrospora osmanthi Mei Wang & L. Cai. (2017) (Figure 2E).

Sexual morph: Undetermined. Asexual morph on SNA: *Hyphae* branched, guttulate, septate, hyaline to pale brown, 1.8–6.4 µm diam. *Conidiophores* mostly reduced to conidiogenous cells. *Conidiogenous cells* monoblastic, discrete, determinate, brown, subspherical, ampulliform to cylindrical, 4.8–15.3 × 4.1–11.9 µm (av. = 8.58 ± 3.28 × 6.34 ± 1.76). *Conidia* solitary, globose or subglobose, aseptate, initially pale brown, becoming black with age, shiny, smooth-walled, sometimes formed directly from the mycelia, 9.9–16.7 µm diam. (av. = 13.07 ± 1.29).

Culture characters on PDA: Colonies flat, floccose, undulate, surface initially white (1A1), becoming grayish green (1D3), abundant aerial mycelium, reverse concolorous with dark patches, reaching 90 mm diameter in 5 d at 25 °C.

Materials examined: South Korea. South Sea, Jeju island, 33°23′53″N, 126°14′24″E, from *Codium* sp. (*Chlorophyta*), 15 August 2021, M. S. Park & Y. W. Lim (SFC20230324-M11, stored in a metabolically inactive state); *ibid*., from *Ulva* sp. (*Chlorophyta*), 15 August 2021, M. S. Park & Y. W. Lim (SFC20230324-M12, stored in a metabolically inactive state) *ibid*., from *Codium* sp. (*Chlorophyta*), 15 August 2021, M. S. Park & Y. W. Lim (SFC20230324-M13, stored in a metabolically inactive state).

Notes: Three *Nigrospora osmanthi* strains (SFC20230324-M11, SFC20230324-M12, and SFC20230324-M13) have bigger conidiogenous cells (5.5–12 µm) and smaller conidia (13.5–16.5 µm) than those of the strains in the original description [7]. Regarding growth on PDA, our isolates achieve a 90 mm diameter on plates within a span of 5 d at 25 °C, whereas the strains in the original description take 10 days to reach the same diameter [7].

5. Discussion

Nigrospora can be identified at the genus level by large dark conidiospores. However, morphological variation sometimes appears in strains despite them belonging to the same species, and different species can share similar morphological characteristics. Therefore, multigenetic marker analysis is imperative for the detection and identification of *Nigrospora*, given that the phylogeny of *Nigrospora* has been well-established using multigenetic markers, including ITS, *BenA*, and *TEF1-α* [7]. A total of nine *Nigrospora* species were identified in this study using multigenetic markers analysis and morphological data. This study provided the first report of five of these species in South Korea (*N. cooperae*, *N. covidalis*, *N. guilinensis*, *N. lacticolonia*, and *N. osmanthi*), and their morphological characteristics are provided in the taxonomy section.

Two *Nigrospora* species, *N. oryzae* and *N. sphaerica*, are frequently encountered. *Nigrospora oryzae* has been consistently reported from macroalgae [16,43] and was also commonly detected in this study on three islands and on three types of algae. However, *Nigrospora sphaerica* was not detected on marine macroalgae in this study, although it has been commonly reported in various marine environments [44,45]. Taritla et al. [18] isolated a strain L18/35 from the macroalga *Sargassum muticum* and identified it as *N. sphaerica*, but we confirmed that the ITS sequence (MF457920) of the strain L18/35 did not match that of *N. sphaerica* provided on NCBI. This creates doubt regarding the viability of its association with macroalga. *Nigrospora aurantiaca* has been reported on sponges [24] but was only isolated from red algae in this study. Until this study, there were no records of *N. cooperae*, *N. covidalis*, *N. guilinensis*, *N. lacticolonia*, *N. osmanthi*, *N. pyriformis*, and *N. rubi* in marine environments. These findings indicate that some *Nigrospora* species can adapt and inhabit both terrestrial and marine habitats, but further research is required to elucidate the underlying mechanisms of such environmental adaptation.

Although secondary metabolites produced by *Nigrospora* isolated from macroalgae have received limited attention in previous studies, numerous valuable secondary metabolites have been isolated from *Nigrospora* species [19]. One such example is nigrosporone B, which has shown anti-cancer, anti-bacterial, cytotoxic, and anti-malarial activities [46]. *Nigrospora aurantiaca* produces a red pigment known as bostrycin that can be used as a natural dye [47], and the same color pigment was observed in our *N. aurantiaca* strains SFC20230324-M01 and SFC20230324-M02 as well. Notably, *Nigrospora* species isolated from marine environments also produce a diverse range of secondary metabolites, many of which exhibit beneficial properties such as antimicrobial, antitumor, and cytotoxic activities [48–50].

The diversity of *Nigrospora* species was found to be highest in brown algae, followed by red algae and green algae. With the exception of *N. oryzae* and *N. pyriformis*, all species were exclusively isolated from a specific algal type (Figure 1). It is too early to conclude that *Nigrospora* species have a symbiotic relationship with algae due to the limited number of studied samples. However, considering that *Arthrinium* spp., a sister genus of *Nigrospora*, improves the survival of brown algae by providing antioxidants in response to decreased photosynthetic activity [51], it is possible that *Nigrospora* may also interact with algae. Moreover, pyrenocines isolated from *Phaeosphaeria* sp. can protect macroalgae against protistan pathogens, such as *Olpidiopsis pyropia* (*Oomycota*), through the collapse of the zoosporangia of *O. pyropia* [52]. Therefore, further investigations are required to elucidate the ecological role of *Nigrospora* associated with macroalgae and whether it acts as an endophyte or a pathogen. This study provides insights and discusses the possibility of biologically meaningful interactions between *Nigrospora* and macroalgae. Further studies aimed at comprehending the role of algicolous *Nigrospora* will greatly contribute to the effective management of macroalgal aquaculture and pathogenicity.

Acknowledgments

We thank Editage (www.editage.co.kr) for English language editing.

Disclosure statement

The authors have declared that no competing interests exist.

Funding

This work was supported by the management of Marine Fishery Bio-resources Center (2023), funded by the National Marine Biodiversity Institute of Korea (MABIK).

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Data availability

All the data that support the findings of this study are available within the article.

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