The genus *Arthrinium* (*Ascomycota, Sordariomycetes, Apiosporaceae*) from marine habitats from Korea, with eight new species

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**Abstract**

Species of *Arthrinium* are well-known plant pathogens, endophytes, or saprobes found in various terrestrial habitats. Although several species have been isolated from marine environments and their remarkable biological activities have been reported, marine *Arthrinium* species remain poorly understood. In this study, the diversity of this group was evaluated based on material from Korea, using morphological characterization and molecular analyses with the internal transcribed spacer (ITS) region, β-tubulin (TUB), and translation elongation factor 1-alpha (TEF). A total of 41 *Arthrinium* strains were isolated from eight coastal sites which represented 14 species. Eight of these are described as new to science with detailed descriptions.

**Keywords:** Fungal diversity, Marine fungi, Multigene phylogeny, Eight new taxa

**INTRODUCTION**

The genus *Arthrinium*, which belongs to *Apiosporaceae* in *Xylariales* (*class Sordariomycetes in Ascomycota*), was first recognized and established more than 200 years ago, with *A. caricicola* as type species (Schmidt and Kunze 1817). To date, it comprises approximately 88 species worldwide (Index Fungorum: http://www.indexfungorum.org).

*Arthrinium* species have traditionally been classified based on morphological characteristics such as conidial shape, conidiophores, and the presence or absence of sterile cells and setae (Schmidt & Kunze 1817; Hughes 1953; Minter 1985). Among these characteristics, conidial shape appears to be diagnostic for distinguishing species (Singh et al. 2013). However, morphological variation is often observed depending on the growth substrate and incubation period (Crous & Groenewald 2013). As such, species identification based on morphological characteristics is problematic and impractical. To address this problem, DNA sequences of the internal transcription spacer (ITS), translation elongation factor 1-alpha (TEF), and β-tubulin gene (TUB) were employed to delimit and recognize closely related *Arthrinium* species and infer their phylogenetic relationships (Crous & Groenewald 2013).

*Arthrinium* species have been globally reported as endophytes, plant pathogens, and saprobes and are commonly isolated from various terrestrial environments, including air, plants, and soil (Kim et al. 2011; Crous & Groenewald 2013; Wang et al. 2018). More recently, isolation from various marine environments, including seawater, seaweed, and the inner tissues of marine sponges,
has been reported (Miao et al. 2006; Tsukamoto et al. 2006; Suryanarayan 2012; Flewelling et al. 2015; Hong et al. 2015; Wei et al. 2016; Elissawy et al. 2017; Li et al. 2017). Arthrinium species isolated from sponges, egg masses of saffin sandfish, and seaweeds showed promising bioactive properties, including high enzymatic activity, antifungal activity, and antioxidant capacity (Elissawy et al. 2017; Li et al. 2017; Park et al. 2018). Some species (A. arundinis, A. pheospermum, A. rasikravindrae, A. sacchari, and A. saccharicola) have been detected in both marine and terrestrial environments (Wang et al. 2018). Whether these species have specific adaptations to survive in seawater requires further investigation. A recent study showed that marine Arthrinium species developed strategies to adapt to marine environments, such as a symbiotic partnership with seaweed (Heo et al. 2018). In marine systems, dissolved organic matter in seawater can absorb ultraviolet radiation and produce reactive oxygen species (ROS), which cause oxidative stress on marine microorganisms (Mopper & Kieber 2000). Heo et al. (2018) detected relatively high antioxidant activity and radical-scavenging activity in marine-derived Arthrinium species. The antifungal activity of seaweed-pathogenic fungi has also been studied (Hong et al. 2015; Heo et al. 2018). Arthrinium saccharicola (KUC21342) has the potential to inhibit the growth of Asteromyces cruciatus, a pathogenic fungus that attacks brown algae (Heo et al. 2018). The discovery of the promising bioactivities of marine Arthrinium species was one of the reasons motivating our subsequent investigation of the diversity of marine Arthrinium in Korea.

Six species of Arthrinium have previously been reported from marine environments in Korea: A. arundinis, A. marii, A. pheospermum, A. rasikravindrae, A. sacchari, and A. saccharicola (Hong et al. 2015; Heo et al. 2018; Park et al. 2018). However, many marine species remain unidentified owing to the lack of resolution in ITS-based phylogenies and the paucity of morphological characteristics. The aim of this study was to investigate marine Arthrinium species from coastal environments in Korea and to identify them using morphological characteristics and multigene phylogenies (ITS, TEF, and TUB).

**MATERIALS AND METHODS**

**Sampling and isolation**

The seaweed Sargassum fulvellum and unidentified seaweeds were collected from two locations, Taean-gun on the west coast of Korea and Jeju Island south of Korea. To isolate the fungi, the seaweeds were washed with distilled water and cut into small pieces (approximately 5 mm diam) using a sterile surgical blade. The pieces were treated with 70% ethanol for 60 s and washed in sterile distilled water for 10 s. Each piece was placed on 2% malt extract agar (MEA) supplemented with 0.01% streptomycin and 0.01% ampicillin to inhibit bacterial growth. The plates were incubated at 25 °C for 7–15 d.

Suspected Arthrinium colonies were transferred onto potato dextrose agar (PDA, Difco, Sparks, MD, USA) plates. The colonies were subsequently identified as belonging to Arthrinium based on ITS sequences (see below). A total of 14 Arthrinium strains were isolated in this study and an additional 27 Arthrinium strains were obtained from the Seoul National University Fungus Collection (SFC), Seoul, Korea. Each strain is stored in 20% glycerol at −80 °C in the Korea University Fungus Collection (KUC), Seoul, Korea. Type specimens were deposited in the Korean Collection for Type Culture, Daejeon, Korea (KCTC), with ex-type living cultures deposited in KUC.

**DNA extraction, PCR amplification, and sequencing**

Genomic DNA was extracted using an Accuprep Genomic DNA extraction kit (Bioneer, Korea) according to the manufacturer’s protocol. PCR targeting the ITS, TUB, and TEF regions was carried out according to a previously described method (Hong et al. 2015). For the ITS region, the primers ITS1F and ITS4/LR3 were used (White et al. 1990; Gardes & Bruns 1993); for TUB, we employed Bt2a/T10 and Bt2b/T2 (Glass & Donaldson 1995; O’Donnell & Cigelnik 1997), and for TEF, we used EF1-728F and EF2 (O’Donnell et al. 1998; Carbone & Kohn 1999). All PCR products were checked on a 1% agarose gel and purified with the AccuPrep PCR/Gel DNA Purification Kit (Bioneer, Seoul, Korea). DNA sequencing was performed at Macrogen (Seoul, Korea) on an ABI3730 automated DNA Sequencer (Applied Biosystems, Foster City, CA) using the same set of primers for each locus. Additional DNA sequences of some strains were obtained from previous studies (Hong et al. 2015; Heo et al. 2018). All new sequences generated in this study were deposited in GenBank (Table 1).

**Phylogenetic analysis**

ITS sequences were assembled, proofread and edited using MEGA v. 7 (Kumar et al. 2016) and subsequently aligned with Arthrinium reference sequences from GenBank using MAFFT 7.130 (Katoh and Standley 2013). To adjust the ambiguous alignment manually, maximum likelihood analysis was performed using all sequence where ambiguous regions excluded using G-block. Then, the original sequences were aligned based on the supported clades, and ambiguous regions were manually adjusted.

Maximum likelihood (ML) analyses were conducted using RAxML v. 7.03 (Stamatakis 2006) and a GTR + G model with 1000 bootstrap replicates. Bayesian tree inference (BI) was carried out using MrBayes version 3.2.
Table 1 A list of all the strains included in the phylogenetic analysis

| Identity                  | Culture no.* | Isolation source            | Location           | GenBank accession no. b |
|---------------------------|--------------|------------------------------|--------------------|-------------------------|
|                           |              |                              |                    | ITS | TUB | TEF |
| **A. agari sp. nov.**     | KUC21333 =   | *Agarum cribrosum* Yangyang-gun, Korea | MH498520 MH498478 MH544663 |
|                           | SFC20161014-M18 |                             |                    |    |     |     |
| KUC21361                  | *Agarum cribrosum* Yangyang-gun, Korea | MH498519 MH498477 MN868914 |
| KUC21362                  | *Agarum cribrosum* Yangyang-gun, Korea | MH498518 MH498476 MN868915 |
| KUC21363                  | *Agarum cribrosum* Yangyang-gun, Korea | MH498517 MH498475 MN868916 |
| KUC21364                  | *Agarum cribrosum* Yangyang-gun, Korea | MH498516 MH498474 MN868917 |
| **A. arctoscopi sp. nov.**| KUC21331 =   | *Egg of Arctoscopus japonicus* Goseong-gun, Korea | MH498528 MH498486 MN868919 |
|                           | SFC20200506-M05 |                             |                    |    |     |     |
| KUC21344                  | *Egg of Arctoscopus japonicus* Goseong-gun, Korea | MH498527 MH498485 MN868920 |
| KUC21345                  | *Egg of Arctoscopus japonicus* Goseong-gun, Korea | MH498526 MH498484 MN868921 |
| KUC21346                  | *Egg of Arctoscopus japonicus* Goseong-gun, Korea | MH498525 MH498483 MN868922 |
| **A. arundinis**          | CBS 124788   | Living leaves of Fagus sylvatica Basel, Switzerland | KF144885 KF144975 KF145017 |
|                           | 114336       | Leaf of Hordeum vulgare      Shabestar, Iran | KF144884 KF144974 KF145016 |
| KUC21261                  | *Sargassum fulvellum* Jeju-do, Korea | KT207779 MH498511 MH544683 |
| KUC21229                  | *Sargassum fulvellum* Jeju-do, Korea | KT207747 MH498512 MH544684 |
| KUC21337                  | *Beach Sand* Muan-gun, Korea | MH498551 MH498509 MH544682 |
| **A. aureum**             | CBS 244.83   | Air                           Barcelona, Spain | AB220251 KF144981 KF145023 |
| **A. balearicum**         | AP24118 = CBS 145129 | Undetermined Poaceae Liucmajor, Spain | MK014869 MK017975 – |
| **A. bambusae**           | LC7106       | Leaf of bamboo                China | KY494718 KY705186 KY806204 |
|                           | LC7107       | Leaf of bamboo                China | KY494719 KY705187 KY705117 |
| **A. camelliae-sinensis** | LC5007       | *Camellia sinensis* China | KY494704 KY705173 KY705103 |
|                           | LC8181       | *Brassica capestris* China | KY494761 KY705229 KY705157 |
| **A. descalsii**          | AP3118A = CBS 145130 | *Ampelodesmos mauritanicus* Spain | MK014870 MK017976 – |
| **A. dichotomanthi**      | LC4950       | *Dichotomanthus tristianaearpa* China | KY494697 KY705167 KY705096 |
|                           | LC8175       | *Dichotomanthus tristianaearpa* China | KY494755 KY705223 KY705151 |
| **A. espeorense**         | AP16717 = CBS 145136 | *Phyllostachys aurea* Spain | MK014878 MK017983 – |
| **A. euphorbiae**         | IMI 285638b  | *Bambusa sp.* Bangladesh | AB220241 AB220288 – |
| **A. fermenti sp. nov.**  | KUC21289 =   | *Seaweed* Haenam-gun, Korea | MF615226 MF615231 MH544667 |
|                           | SFC20140423-M86 |                             |                    |    |     |     |
| KUC21288                  | *Seaweed* Haenam-gun, Korea | MF615230 MF615235 MH544668 |
| **A. gaoyouense**         | CFCC 52301   | *Phragmites australis* China | MH197124 MH236789 MH236793 |
|                           | CFCC 52302   | *Phragmites australis* China | MH197125 MH236790 MH236794 |
| **A. gartenyi**           | JHB9004 = HKAS96289 | *Culms of dead bamboo* China | KY356086 – – |
| **A. guizhouense**        | LC5318       | *Air in karst cave* China | KY494708 KY705177 KY705107 |
|                           | LC5322       | *Air in karst cave* China | KY494709 KY705178 KY705108 |
| **A. gutiae**             | CBS 135835   | *Gut of a grasshopper* India | K9011352 K9011350 K9011351 |
| **A. hispanicum**         | IMI 326877   | *Maritime sand* Spain | AB220242 AB220289 – |
| **A. hydei**              | CBS 114990   | *Culms of Bambusa tuloides* Tai Po Kau, Hong Kong | KF144890 KF144982 KF145024 |
| JHB90012 = HKAS96355      | *Dead culms of bamboo* China: Kunming | KY356087 – – |
| LC7103                    | *Leaf of bamboo* China | KY494715 KY705183 KY705114 |
| LC7105                    | *Leaf of bamboo* China | KY494717 KY705185 KY705116 |
| Identity       | Culture no. a | Isolation source                   | Location                | GenBank accession no. b |
|---------------|---------------|-----------------------------------|-------------------------|-------------------------|
|               |               |                                   | **ITS** | **TUB** | **TEF** |
| A. hyphopodii | MFLUCC 15–0003 | Culms of *Bambusa tuloides*       | Thailand               | KR069110                | –      |
|               | JHB003 = HKAS96288 | Culms of Bamboo                  | China; Kunming         | KY350688                | –      |
| A. hysterinum | CBS 145133    | *Phyllostachys aurea*             | Spain                  | MK014875                | MK017981 |
|               | CBS 145135    | *Phyllostachys aurea*             | Spain                  | MK014877                | MK017982 |
| A. ibericum   | AP10118 T = CBS 145137 | *Arundo donax*                  | Portugal               | MK014879                | MK017984 |
| A. italicum   | AP221017 T = CBS 145138 | *Arundo donax*                  | Italy                  | MK014880                | MK017985 |
|               | AP29118 = CBS 145139 | *Phyllostachys aureal*           | Spain                  | MK014881                | MK017986 |
| A. jiangxiense| LC2831        | Leaf of bamboo                    | China                  | KY494686                | KY806201 |
|               | LC4494        | *Phyllostachys sp.*               | China                  | KY494690                | KY705160 |
| A. kogelbergense | CBS 113332 | Culms of *Cannomois virgata*     | Republic of South Africa | KF144891                | KF144983 |
|               | CBS 113333 | Dead culms of Restionaceae       | Republic of South Africa | KF144892                | KF144984 |
| A. koreanum sp. nov. | KUC21332 T = SFC20200506-M06 | Egg of *Arctoscopus japonicus* | Goseong-gun, Korea   | MH498524                | MH498542 |
|               | KUC21348     | Egg of *Arctoscopus japonicus*    | Goseong-gun, Korea     | MH498523                | MH498481 |
|               | KUC21349     | Egg of *Arctoscopus japonicus*    | Goseong-gun, Korea     | MH498522                | MH498480 |
|               | KUC21350     | Egg of *Arctoscopus japonicus*    | Goseong-gun, Korea     | MH498521                | MH498479 |
| A. longistromum | MFLUCC 11–0481 | Culms of Decaying bamboo       | Thailand               | KU940141                | –      |
|               | MFLUCC 11–0479 | Culms of Decaying bamboo       | Thailand               | KU940142                | –      |
| A. malaysianum | CBS 251.29  | Stem base of *Cinnamomum camphara* | Malaysia              | KF144897                | KF144989 |
|               | CBS 102053   | *Macaranga hulletii* stem colonized by ants | Gombak, Malaysia  | KF144896                | KF144988 |
| A. marii      | KUC21338 = SFC20140423-M01 | Seaweed                          | Muan-gun, Korea       | MH498549                | MH498507 |
|               | CBS 113535   | Oats                             | Sweden                 | KF144898                | KF144990 |
|               | CBS 114803   | Culm of *Arundinaria hindsii*    | Lung Fu Shan, Hong Kong | KF144899                | KF144991 |
| A. marinum sp. nov. | KUC21328 T = SFC20140423-M02 | Seaweed                          | Suncheon-si, Korea    | MH498538                | MH498496 |
|               | KUC21353     | Seaweed                          | Suncheon-si, Korea     | MH498537                | MH498495 |
|               | KUC21354     | Seaweed                          | Suncheon-si, Korea     | MH498536                | MH498494 |
|               | KUC21355     | Seaweed                          | Suncheon-si, Korea     | MH498535                | MH498493 |
|               | KUC21356     | Seaweed                          | Suncheon-si, Korea     | MH498534                | MH498492 |
| A. mediterranei | IMI 326875 | Air                              | Spain                  | AB220243                | AB220290 |
| A. mytilomorphum | DAOI 214595 | Dead blades of *Andropogon sp.* | India                  | KY494685                | –      |
| A. obovaturn  | LC4940       | *Lithocarpus* sp.                | China                  | KY494696                | KY705166 |
|               | LC8177       | *Lithocarpus* sp.                | China                  | KY494757                | KY705225 |
| A. ovatum     | CBS 115042   | *Arundinaria hindsii*            | Hong Kong              | KF144903                | KF144995 |
| A. phaeospermum | KUC21339    | *Phragmites australis*           | Boseong-gun, Korea    | MH498550                | MH498508 |
|               | CBS 114314   | Leaf of *Hordeum vulgare*        | Marand, Iran           | KF144904                | KF144996 |
|               | CBS 114315   | Leaf of *Hordeum vulgare*        | Shabestar, Iran        | KF144905                | KF144997 |
| A. phragmitis | CPC 18900    | *Phragmites australis*           | Bormaro, Italy         | KF144909                | KF145001 |
| A. piptatheri | AP4817A T = CBS | *Piptatherum milicaleum*      | Spain                  | MK014893                | –      |

[1] Kwon et al. *IMA Fungus* (2021) 12:13
| Identity            | Culture no. | Isolation source          | Location                | GenBank accession no. |
|---------------------|-------------|----------------------------|-------------------------|-----------------------|
|                     |             |                            |                         | ITS                   |
|                     |             |                            |                         | TUB                   |
|                     |             |                            |                         | TEF                   |
| **A. pseudoparenchymaticum** |             |                            |                         |                       |
|                     | KUC21279    | *Sargassum fulvellum*      | Jeju-do, Korea          | KT207736              |
|                     |             |                            |                         | KT207636              |
|                     | LC7234      | Leaf of bamboo             | China                   | MF615229              |
|                     | LC8173      | Leaf of bamboo             | China                   | MF615234              |
| **A. pseudosinense** |             |                            |                         |                       |
|                     | CPC 21546   | *Leaf of bamboo*           | Jeju-do, Korea          | KT207736              |
|                     |             |                            |                         | KT207636              |
| **A. pseudospagazzinii** |             |                            |                         |                       |
|                     | CBS 102052  | *Mocaranga hulletii* stem  | Gombak, Malaysia        | KT207736              |
|                     |             | colonized by ants          |                         | KT207636              |
| **A. pterospermum**  |             |                            |                         |                       |
|                     | CPC 20193   | *Lepidosperma gladiatum*   | Adelaide, Australia     | KT207736              |
|                     | CBS 123185  | *Mochaerina sinclairii*    | Auckland, New Zealand  | KT207736              |
| **A. pusillospermum sp. nov.** |             |                            |                         |                       |
|                     | KUC21321 T  | *Seaweed*                  | Taean-gun, Korea       | MH498533              |
|                     |             |                            |                         | MH498491              |
|                     | KUC21357    | *Seaweed*                  | Taean-gun, Korea       | MH498532              |
|                     |             |                            |                         | MH498490              |
| **A. qinlingense**   | CFCC 52303  | *Fargesia qinlingensis*    | China                   | MH197120              |
|                     | CFCC 52303  | *Fargesia qinlingensis*    | China                   | MH197121              |
| **A. rasikravindrae** |             |                            |                         |                       |
|                     | CBS 337.61  | *Cissus sp.*               | Netherlands             | KT207736              |
|                     |             |                            |                         | KT207636              |
| **A. pseudospegazzinii** |             |                            |                         |                       |
|                     | CPC 21602   | *Rice*                     | Thailand                | KT207736              |
|                     | LC5449      | Soil in karst cave         | China                   | KT207736              |
|                     | LC7115      | Leaf of bamboo             | China                   | KT207736              |
| **A. sacchari**      | KUC21340 =  | *Egg of Arctoscopus japonicus* | Goseong-gun, Korea | MH498534              |
|                     | SFC20200506-M04 |                         |                         | MH498547              |
|                     | CBS 301.49  | *Bamboo*                   | Indonesia               | KT207736              |
|                     | CBS 212.30  | *Phragmites australis*     | Cambridge, United Kingdom | KT207736          |
|                     | CBS 372.67  | *Air*                      | –                       | KT207736              |
| **A. saccharicola**  | KUC21221    | *Sargassum fulvellum*      | Hyeopjae Beach, Jeju-do | KT207736              |
|                     |             |                            |                         | KT207637              |
|                     | KUC21342 =  | *Egg of Arctoscopus japonicus* | Goseong-gun, Korea | MH498546              |
|                     | SFC20160407-M06  |                         |                         | MH498547              |
|                     | KUC21343 =  | *Egg of Arctoscopus japonicus* | Yeongok-myeon, Gangneung-si | MH498545              |
|                     | SFC20161110-M12 |                         |                         | MH498530              |
|                     | CBS 191.73  | *Air*                      | Utrecht, Netherlands    | MH498545              |
|                     | CBS 463.83  | Dead culms of Phragmites australis | Harderboes, Netherlands | MH498547              |
| **A. sargassi sp. nov.** |             |                            |                         |                       |
|                     | KUC21228 T  | *Sargassum fulvellum*      | Jeju-do, Korea          | KT207736              |
|                     |             |                            |                         | KT207644              |
|                     | KUC21232    | *Sargassum fulvellum*      | Jeju-do, Korea          | KT207750              |
|                     |             |                            |                         | KT207648              |
|                     | KUC21284    | *Sargassum fulvellum*      | Jeju-do, Korea          | KT207750              |
|                     |             |                            |                         | KT207648              |
|                     | KUC21287    | *Sargassum fulvellum*      | Jeju-do, Korea          | KT207750              |
|                     |             |                            |                         | KT207648              |
| **A. serenense**     | IMI 326869  | Food, pharmaceutical excipients, atmosphere | Spain             | AB220250              |
|                     |             |                            |                         | AB220297              |
| **A. subroseum**     | LC7215      | *Leaf of bamboo*           | China                   | KT207736              |
|                     | LC7291      | *Leaf of bamboo*           | China                   | KT207736              |
| **A. taeanense sp. nov.** |             |                            |                         |                       |
|                     | KUC21322 T  | *Seaweed*                  | Taean-gun, Korea       | MH498515              |
|                     |             |                            |                         | MH498473              |

Kwon et al. IMA Fungus (2021) 12:13 Page 5 of 26
Table 1 A list of all the strains included in the phylogenetic analysis (Continued)

| Identity | Culture no. | Isolation source | Location | GenBank accession no. |
|----------|-------------|------------------|----------|-----------------------|
| A. thailandicum | MFLUCC 15–0202 | Culms of Dead bamboo | Thailand | KU940145  –  –  |
|          | LC5630      | Rotten wood       | China    | KYY49714  KYY806200  KYY705113 |
| A. vietnamense | IMI 99670   | *Citrus sinensis* | Vietnam  | KX986996  KYY019466  –  |
| A. xenocordella | CBS 478.86 | Soil              | Matopos, Zimbabwe | KF144925  KF145013  KF145055 |
|          | LC3486      | *Camellia sinensis* | China    | KYY49687  KYY705158  KYY705086 |
| A. yunnanum | MFLUCC 15–0002 | Culms of Decaying bamboo | China | KU940147  –  –  |
|          | DDQQ0028    | *Phyllostachys nigra* | China    | KU940148  –  –  |
| Nigrospora gorlenkoana | CBS 480.73 | *Vitis vinifera* | Kazakhstan | KX986048  KYY019456  KYY019420 |

Notes: * indicates ex-type

CBS: Culture Collection of M. F. L. (MFLUCC) at the Chinese Academy of Sciences, Kunming, China;
KFCC: Korea Fungal Culture Collection, Daegu, Korea;
NKFC: National Fungal Culture Collection of India;
LC: Laboratory Culture Collection of Faculty of Agriculture, Kyung Hee University, South Korea;
CABI: CABI Bioscience, Eggham, UK;
CPC: Culture collection of J. B. Cai, Chinese Academy of Sciences, Kunming, China;
CFCC: Chinese Forestry Culture Collection Centre, Beijing, China;
HKAS: Herbarium of the Royal Akademie van Wetenschappen, Brussels, Belgium;
CAS: Chinese Academy of Sciences, Kunming, China;
DAOM: Canadian Collection of Fungal Cultures, Ottawa, Canada;
HMAS: Herbarium of Minnesota State University, Mankato, MN, USA;
NCIMB: National Collection of Industrial and Marine Bacteria, Aberdeen, UK;
MNHU: Max-Planck Institute of Molecular Plant Physiology, Potsdam, Germany;
NBRC: National Institute of Agrobiological Resources, Fuji, Japan;
NIGR: National Institute of Genomic Research, New Delhi, India;
NMF: Natural Microbial Facilty, Rikkyo University, Tokyo, Japan;
NMBU: Norwegian University of Life Sciences, Asker, Norway;
DBFZ: Deutsches Botanisches Forschungszentrum, Gatersleben, Germany;
CABI: CABI Bioscience, UK;
BCRC: Bioculture Resource Collection, Taiwan, ROC;
NFCCI: National Fungal Culture Collection of India;
KIB: Korea Institute of Bioscience and Biotechnology, Daejeon, Korea;
KU: Korea University Fungus Collection, Seoul, Korea;
BCRC: Bioculture Resource Collection, Taiwan, ROC;
LC3486 Carolina Seeds, USA;
DDQ: NCMB, Denmark;
MH498513 MH498471 MN868935

The sequences generated in this study are shown in bold

(Concluded)

(Ronquist et al. 2012), with the best model (HKY + I + G) selected for each marker based on the Bayesian information criteria using jModeltest v. 2.1.10 (Darriba et al. 2012). To achieve stationary equilibrium, 20 million trees were generated, and trees were sampled every 1000 generations. The first 25% of the trees was discarded as burn-in, and the remaining 75% was used for calculating posterior probabilities (PP) in the majority rule consensus tree. All analyses were performed on the CIPRES web portal (Miller et al. 2010).

The sequences of the other two loci (TEF and TUB) were individually aligned with *Arthrinium* reference sequences from GenBank using the same approach described for the ITS. ML and BI analyses also followed the above criteria. The models for TEF and TUB were HKY + I + G and K80 + I + G, respectively. The ITS taxa for the multigene tree were different from those of the above criteria. The models for TEF and TUB were HKY + I + G and K80 + I + G, respectively. The ITS taxa for the multigene tree were different from those of the single ITS tree, so the model test for the ITS region was redone for the multigene analysis. As a result, the SYM + G model was applied to ITS region in the multigene tree. Finally, sequence concatenation was performed using the same methods and models assigned for each locus described above.

Morphological observation

Strains were grown on oatmeal agar (OA, Difco™), PDA, and MEA at 15, 20, and 25°C in darkness for 14 d. The culture characteristics, such as surface structure, presence of aerial mycelium and the colour of the mycelium, colour of colony or medium, and sporulation (Crous et al. 2009), were recorded. Colors and the corresponding codes were evaluated according to the Munsell color chart (Munsell Color, 2009). To determine fungal growth rates, the diameter of each colony was measured every 24 h, and each measurement was performed in triplicate. Microscopic characters were observed with an Olympus BX51 light microscope (Olympus, Tokyo, Japan). Samples were mounted in water to take pictures of conidiophores and conidia, and pictures were taken using a DP20 microscope camera (Olympus, Tokyo, Japan). At least 30 individuals were measured for each microscopic character. To illustrate the range of variation, 5% of the extreme measurements from each end of the range are given in parentheses.

Scanning electron microscope (SEM) was used to observe detailed morphological characters. Colonies sporulating abundantly on PDA, MEA, and OA were freeze-dried. Ion coating and observation were performed by Wooyoung Solution Inc. (Suwon, Korea), using an S-5200 scanning electron microscope (Hitachi, Tokyo, Japan). The SEM images were taken under 1500x to 8000x magnifications.

RESULTS

A total of 41 *Arthrinium* strains were identified, representing six known and eight new species. Of these strains, 26 were isolated from various seaweeds, 14 from the eggs of sailfin sandfish, and one from beach sand. The dominant species were three of the new species, *A. arctoscopi* (5 strains), *A. arctoscopi* (5 strains), and *A. mari- num* (5 strains) (Table 1).

A total of 21 ITS (580–1150 bp), 24 TEF (420–970 bp), and 22 TUB (400–560 bp) sequences were newly generated for the 41 *Arthrinium* strains. The ITS phylogeny contained 124 terminals, including *Nigrospora gorlenkoana* as outgroup. The concatenated three-gene phylogeny contained 95 terminals, consisting of 749, 613, and 503 characters respectively, including gaps.
Preliminary identification was based on the ITS region, and multigene analysis was used to test the identifications, determine the phylogenetic relationships among the taxa, and to resolve closely related species. Both the ML and Bayesian analyses showed the same tree topologies and the ML tree is represented (Figs. 1, 2). ML and Bayesian analyses showed the same tree topology, determine the phylogenetic relationships among the taxa, and to resolve closely related species. Both the multigene analysis was used to test the identification, and the ML tree is represented (Figs. 1, 2).

The 41 Arthrinium strains obtained in this study formed five clades (A, B, C, D, and E), both in the ITS-based and combined phylogeny analyses (Figs. 1, 2). In the ITS tree, many Arthrinium species were distinguished from one another. However, some were not clearly separated (clades B and D) and the relationships of the others (clades C and D) were not resolved. The above problem was solved in the individual trees of TEF and TUB (Figs. 1S, 2S), and the multigene tree based on the ITS, TUB, and TEF regions (Fig. 2). The multigene analysis supported the conclusion that six taxa corresponded to known species. Eight putatively novel species were classified into five clades (Fig. 2). The eight species were clearly separated from the previously sequenced taxa, each forming a clade with high support (over 99% of BS, 0.99 of PP) (Fig. 2). Arthrinium agari and A. korcanum. Were included in clade A, A. piptatheri and A. fermenti were in clade D, and A. pusilluspermum and A. taeanense were in clade E. Comparison with morphoanatomical and other data of species that have so far not been sequenced supported our interpretation of these eight entities representing novel species.

**TAXONOMY**

**Arthrinium agari** S.L. Kwon, S. Jang & J.J. Kim, sp. nov.

Mycobank MB834592

(Fig. 3)

*Etymology*: ‘agari’ refers to the generic name of *Agarum cribrosum*, the source of the type strain.

*Molecular diagnosis*: Arthrinium agari is distinguished from the phylogenetically most closely related species, *A. arundinis*, by unique single nucleotide polymorphisms in the three loci used in this study (Figs. 3S, 4S, 5S): ITS positions 21 (C), 31 (indel), 36 (C), 38 (T), 93 (C), 111 (C), 113 (T), 122–124 (indel), 190–203 (indel), 205 (indel), 214–223 (indel), 227 (G), 228 (A), 253 (G), 259 (A), 291 (A), 535 (T), and 645 (indel); TEF positions 14 (A), 16 (G), 17 (T), 32 (C), 35 (A), 47 (C), 54 (T), 59–62 (indel), 64 (T), 65 (T), 79 (G), 85 (G), 96 (T), 125 (G), 135 (indel), 151 (C), 173 (G), 174 (A), 176 (G), 192 (T), 213 (C), 249 (G), 265 (C), 271 (C), 288 (G), 302 (T), 306 (G), 312 (indel), 331 (G), and 494 (A); TUB positions 15 (G), 29 (A), 31 (A), 62 (T), 67 (G), 80 (T), 89 (A), 98 (G), 99 (C), 138 (T), 139 (T), 143 (T), 199 (T), 208 (A), 210 (A), 212 (A), 223 (T), 229 (A), 232 (T), 312 (C), 324 (A), 331 (G), 377 (T), 428 (C), 467 (T), and 482 (A).

*Type*: **Korea**: Gangwon-do, Yangyang-gun, 38°07′04.8″N, 128°38′00.8″E, isolated from *Agarum cribrosum*, 11 Sept. 2016, *M.S. Park* (Herb. KCTC 46909 – holotype preserved in a metabolically inactive state; KUC21333 = NIBRFGC000501588, SFC20161014-M18 – ex-type cultures).

*Description*: Mycelium of smooth, hyaline, branched, septate, hyphae 2.0–3.5 μm diam. Conidiogenous cells aggregated in clusters on hyphae or solitary, at first hyaline, becoming pale green, cylindrical, sometimes ampulliform. *Conidia* brown, smooth to granular, globose to subglobose in surface view, (8.5–)9.0–10.5 × (7.0–)7.5–8.5 (–9.0) μm (μ = 9.5 × 8.1 μm, n = 30); lenticular in side view, with equatorial slit, 5.5–7.0 μm wide (μ = 6.4 μm, n = 30), elongated cell observed.

*Culture*: PDA: colonies thick, concentrically spreading with aerial mycelium, margin irregular; mycelia white to grey and pale brown coloured; colour diffused in media; odour indistinct. MEA: colonies low, flat, concentrically spreading with sparse aerial mycelium, margin circular; mycelia white; sporulation not observed; pigment absent in medium; odour indistinct. OA: colonies thick, concentrically spreading with aerial mycelium, margin circular; mycelia white to pink; sporulation was not observed; partially pink (2.5YR 8/3) pigment diffused in media; odour indistinct. Colony diameters (in mm after 120 h): 15°C PDA 19–20, MEA 15–18, OA 11–13; 20°C PDA 34–35, MEA 28–34, OA 20–23; 25°C PDA 24–28, MEA 22–25, OA 19–20.

*Additional material examined*: **Korea**: Gangwon-do, Yangyang-gun, 38°07′04.8″N, 128°38′00.8″E, isolated from *Agarum cribrosum*, 11 Sept. 2016, *M.S. Park* (KUC21361, KUC21362, KUC21363, and KUC21364).

*Notes*: Arthrinium agari is phylogenetically related to *A. arundinis* (over 97.52% similarity in the ITS region, 93.74% in the TEF region, and 93.64% in the TUB region) (Figs. 1, 2). The two species also morphologically resemble each other. The two species have smooth, hyaline, branched, septate mycelium, and ampulliform conidiogenous cells that cluster on hyphae. *Arthrinium arundinis* and *A. agari* have similar conidia shape (brown, globose in surface view, lenticular in side view) (Crous & Groenewald 2013). However, *A. agari* can be distinguished from *A. arundinis* by its larger conidia (*A. agari*: 8.5–10.5 × 7.0–9.0 μm, *A. arundinis*: 5–6 × 3–4 μm diam) (Crous & Groenewald 2013).

*Arthrinium agari* and *A. sinensis* (non-sequenced species) also have similar conidia shape (globose in surface view, lenticular in side view). However, they can be distinguished by the shape of the conidiogenous cell; cylindrical and sometimes ampulliform in *A. agari*, whereas lageniform in *A. sinensis* (Table 2).

*Arthrinium arctoscopi* S.L. Kwon, S. Jang & J.J. Kim, sp. nov.

Mycobank MB834593
Etymology: ‘arctoscopi’ refers to the generic name of *Arctoscopus japonicus*, the substrate of which it was found.

Molecular diagnosis: *Arthrinium arctoscopi* is distinguished from phylogenetically most closely related species, *A. obovatum*, by unique single nucleotide polymorphisms in the three loci used in this study (Figs. 3S, 4S, 5S): ITS positions 112–124 (indel), 128–137 (indel), 190 (indel), 192 (G), 223 (T), 225 (indel), 226 (indel), 253–254 (indel), 618 (G), 621 (C), 624 (C), and 651 (G); TEF positions 32 (T), 33 (T), 76 (G), 131 (C), 132 (C), 145 (T), 148–150 (indel), 207 (indel), 208 (T), 210 (T), 211 (T), 269 (G), 304 (A), 305 (C), 316 (C), 320 (C), 324 (A), 328 (T), and 333 (A); TUB position 5 (T), 8 (C), 27 (G), 38 (T), 53 (G), 62 (A), 68 (C), 79 (C), 80 (A), 82 (G), 87 (T), 90 (A), 106 (A), 112 (T), 144 (A), 211 (indel), 212 (T), 225 (T), 227 (C), 311 (T), 334 (T), 467 (C), 479 (C), and 506 (C).

Type: *Korea*: Gangwon-do, Goseong-gun, 38°28′44.0″N, 128°26′18.9″E, isolated from Egg masses of *Arctoscopus japonicus*, 10 Nov. 2016, M.S. Park (Herb. KCTC 46907 – holotype preserved in a metabolically inactive state).

(Fig. 1) ML tree based on the ITS region. The numbers at the nodes indicate ML bootstrap support (BS) > 75% and Bayesian posterior probabilities (PP) > 0.75 as BS/PP. The thickened branches indicate support greater than 85% for BS and 0.95 for PP. A hyphen (‘-’) indicates values of BS < 70% or PP < 0.75. Ex-holotype strains are indicated with asterisks (**). The fungal cultures examined in this study are shown in bold. Red boxes indicate the novel species. The numbers in the brackets indicate strain number. The scale bar indicates the nucleotide substitutions per position.

(Fig. 2) ML tree based on the ITS, TUB, and TEF regions combined. The numbers at the nodes indicate ML bootstrap support (BS) > 75% and Bayesian posterior probabilities (PP) > 0.75 as BS/PP. The thickened branches indicate support greater than 85% for BS and 0.95 for PP. A hyphen (‘-’) indicates values of BS < 70% or PP < 0.75. Ex-holotype strains are indicated with asterisks (**). The fungal cultures examined in this study are shown in bold. Red boxes indicate the novel species. The numbers in the brackets indicate strain number. The scale bar indicates the nucleotide substitutions per position.
state; KUC21331 = NIBRFGC000501586, SFC20200505- M05 –ex-type cultures).

*Descriptions:* Mycelium of smooth, hyaline, branched, septate, hyphae 2.5–4.0 μm diam. Conidiogenous cells aggregated in clusters on hyphae or solitary, at first hyaline, becoming pale green, cylindrical, sometimes ampulliform. Conidia brown, smooth to granular, globose to elongate ellipsoid in surface view, (9.5–)10–12 (–13) x (7.5–)8.0–11 (–12) μm (\( \bar{x} = 11.1 \times 10 \mu m, n = 30 \)); lenticular in side view, with equatorial slit, 5.5–7.5 μm wide (\( \bar{x} = 6.5 \mu m, n = 30 \)), elongated cell observed.

*Culture:* PDA: colonies thick, concentrically spreading with aerial mycelium, margin irregular; mycelial creamy white; sporulation was not observed; pigment absent in medium; odour indistinct. MEA: colonies flat, concentrically spreading with aerial mycelium, margin irregular;
| Species                  | Habitat         | Isolation source       | Country | Conidia in surface view | Shape | Diam (μm) | Conidia in side view | Shape | Diam (μm) |
|-------------------------|-----------------|------------------------|---------|-------------------------|-------|-----------|----------------------|-------|-----------|
| *A. aureum*             | A               | Airborn spore          | ES      | globose                 |       | 10–30 x 10–15 | –                    | –     | –         |
| *A. guizhouense*        | A               | Airborn spore          | CN      | globose to elongate ellipsoid | 5–7.5 x 4–7 | –          | –                    | –     | –         |
| *A. mediterranei*       | A               | Airborn spore          | ES      | lentiform               |       | 9–9.5 x 7.5–9 | –                    | –     | –         |
| *A. serenense*          | A               | Airborn spore          | ES      | –                      | 10–11 x 8–9.5 | –          | –                    | –     | –         |
| *A. hispanicum*         | M               | Beach sand             | ES      | globose to ellipsoid    |       | 7.5–8.5 x 6–7.5 | lenticular | 6.5   |           |
| *A. agari*              | M               | Costariaceae           | KR      | globose to elongate ellipsoid | 8.5–10.5 x 7–9 | lenticular | 5.5–7                |       |           |
| *A. arctoscopi*         | M               | Egg of Arctoscopus japonicus | KR      | globose to elongate ellipsoid | 9.5–13 x 7.5–12 | lenticular | 5.5–7.5             |       |           |
| *A. koreanum*           | M               | Egg of *A. japonicus*  | KR      | globose to ellipsoid    | 7.5–11 x 5.5–10 | lenticular | 4–6.5                |       |           |
| *A. algicola*           | M               | Sargassaceae           | UA      | globose to ellipsoid    | 10.5–15 x 6–8 | –          | –                    | –     | –         |
| *A. sargassi*           | M               | Sargassaceae           | KR      | globose to elongate ellipsoid | 8.5–11.5 x 8–11 | lenticular | 5.5–7.5             |       |           |
| *A. fermenti*           | M               | Seaweed                | KR      | globose to elongate ellipsoid | 7.5–9 x 7–9 | lenticular | 6–7                  |       |           |
| *A. marinus*            | M               | Seaweed                | KR      | globose to elongate ellipsoid | 9.5–13 x 7.5–10 | lenticular | 6–7                  |       |           |
| *A. pusillospermum*     | M               | Seaweed                | KR      | globose to subglobeobse, elongate cell | 4–6.5 x 3–5.5 | lenticular | 3.5–4.5              |       |           |
| *A. taeanense*          | M               | Seaweed                | KR      | globose to elongate ellipsoid | 5–7 x 4–6 | lenticular | 4–5                  |       |           |
| *A. saccharicola*       | M/P             | Egg of *A. japonicus*  | KRV/ NL | globose to ellipsoid    | (7–8)–9(–10) | lenticular | (4–)5(–6)          |       |           |
| *A. sacchari*           | M/P             | Egg of *A. japonicus*  | UK/ KR  | globose                 | (6–)7(–)     | lenticular | (3.5–)4             |       |           |
| *A. rasikravindrae*     | M/P             | Egg of *A. japonicus*  | KRV/ CN | globose to ellipsoid    | 7–9.5 x 6.5–9 | lenticular | 5–6.5                |       |           |
| *A. arundinis*          | M/P             | Sargassaceae/ Poaceae  | IR/ KR  | globose                 | (5–)6–7     | lenticular | 3–4                  |       |           |
| *A. piptatheri*         | M/P             | Sargassaceae/ Poaceae  | KRV/ ES | globose to elongate ellipsoid | 7.5–10 x 7–9 | lenticular | 4.5–6                |       |           |
| *A. marii*              | M/P             | Seaweed/ Poaceae       | KRV/ HK | globose to elongate ellipsoid | 8–10(–13) | lenticular | (5–)6(–8)          |       |           |
| *A. sporophleum*        | P               | Poaceae                | DE      | fusiform                | 11–14 x 6–8 | –          | –                    | –     | –         |
| *A. descalsii*          | P               | Poaceae                | ES      | globose to ellipsoid    | (5–)7(–)     | lenticular | 6–7                  |       |           |
| *A. mytilomorphum*      | P               | Poaceae                | IN      | fusiform or navicular  | 20–30 x 6–8.5 | –          | –                    | –     | –         |
| *A. ovatum*             | P               | Poaceae                | HK      | oval to boldly ellipsoid | 18–20     | –          | 12–14                |       |           |
| *A. ibericum*           | P               | Poaceae                | PT      | globose to ellipsoid    | (9–)10(–12) | lenticular | (6–)7(–)            |       |           |
| *A. italicum*           | P               | Poaceae                | IT, ES  | globose                 | 4–6 x 3–4   | –          | lenticular            |       |           |
| *A. hydei*              | P               | Poaceae                | CN      | globose                 | (15–)17–19(–22) | lenticular | 11–12                |       |           |
| *A. bambusae*           | P               | Poaceae                | CN      | subglobeobse to ellipsoid | 11.5–15.5 x 7–14 | –          | –                    | –     | –         |
| *A. jiangxiense*        | P               | Poaceae                | CN      | globose to ellipsoid, granular | 7.5–10     | lenticular | 4.5–7                |       |           |
| *A. neogarethjonesii*   | P               | Poaceae                | CN      | globose to subglobeobse | 20–35 x 15–30 | –          | –                    | –     | –         |
| *A. pseudoparenchymaticum* | P              | Poaceae                | CN      | globose to subglobeobse | 13.5–27 x 12–23.5 | –          | –                    | –     | –         |
| *A. pseudosinense*      | P               | Poaceae                | NL      | ellipsoid               | 8–10 x 7–10 | –          | 7–8                  |       |           |
| *A. setostromum*        | P               | Poaceae                | CN      | subglobeobse to obovoid | 18–20 x 15–19 | –          | –                    | –     | –         |
| *A. suberosum*          | P               | Poaceae                | CN      | globose to subglobeobse, ellipsoid | 12–17.5 x 9–16 | –          | –                    | –     | –         |
| *A. thailandicum*       | P               | Poaceae                | CN/ TH  | globose to elongate ellipsoid | 5–9 x 5–8  | lenticular | –                    |       | –         |
| *A. longistromum*       | P               | Poaceae                | TH      | asexual morph: Undetermined | –          | –          | –                    | –     | –         |
| *A. neosubglobosa*      | P               | Poaceae                | CN      | asexual morph: Undetermined | –          | –          | –                    | –     | –         |
**Table 2** Summary of conidial morphology of *Arthrinium* species. Newly established species in this study are shown in bold.

| Species | Habitat | Isolation source | Country | Conidia in surface view | Conidia in side view |
|---------|---------|------------------|---------|-------------------------|---------------------|
|         |         |                  |         | Shape                   | Diam (μm)           | Shape         | Diam (μm) |
| A. subglobosa a | P | Poaceae | TH | asexual morph: Undetermined. | – | – |
| A. macrosorum b | P | Poaceae | CN | – | 17–27 | – | – |
| A. paraphaeospermum c | P | Poaceae | TH | globose to ellipsoid | 10–19 | lenticular | – |
| A. hyphodii d | P | Poaceae | TH | globose to subglobose | 5–10 × 4–8 | – | – |
| A. chinense e | P | Poaceae | CN | subglobose to lenticular | 8.5–12 × 5.5–9 | – | – |

**Continued**
Table 2: Summary of conidial morphology of Arthrinium species. Newly established species in this study are shown in bold (Continued)

| Species | Habitat | Isolation source | Country | Conidia in surface view | Conidia in side view |
|---------|---------|------------------|---------|-------------------------|----------------------|
| A. pterospermum | P | Cyperaceae | AU, NZ | finely roughened irregular | – |
| A. cuspidatum | P | Cyperaceae, Junceae | CA, IN, US, ZA | horn-like tips (tips size: 7 μm) | 21.5 × 10 |
| A. jatrophae | P | Euphorbiaceae | IN | spherical | 65–95 |
| A. pseudopogaggizini | P | Euphorbiaceae | MY | globose | (7–8–9) |
| A. obovatum | P | Fagaceae | CN | obvoid, elongated to ellipsoidal | 11–16/16–31 × 9–16 |
| A. gutta | P | Fagaceae | IT | drop-shaped, oval | 9–12 × 7–11 |
| A. sphærospermum | P | Iridaceae, Myrtaceae, Poaceae | FR | spherical or subspherical | 7–8 |
| A. ushuanense | P | Juncaceae | AR | fusiform or navicular | 17–25 × 6–9 |
| A. luzulae | P | Juncaceae | CH | curved with horn-like tips | 18–21 × 12–14 |
| A. malaysianum | P | Lauraceae, Euphorbiaceae | MY | globose | 5–6 |
| A. kogelbergense | P | Restionaceae | ZA | globose to ellipsoid | 9–10 × 7–8 |
| A. dictyothamthi | P | Rosaceae | CN | globose to subglobose | 9–15 × 6–12 |
| A. vietnamense | P | Rutaceae | VN | globose | 5–6 |
| A. xenocordella | P | Theaceae | CN | globose to somewhat ellipsoid | 9–10 |
| A. aquaticum | P | unknown | CN | globose to subglobose | 9–11 × 8–10 |
| A. scriptum | P | unknown | DE | egg-shape, pear-shape | – |
| A. urticae | P | unknown | IN, TR, CU, BE | subspherical | 4–6 × 3–4 |
| A. gutiae | I | Gut of a grasshopper | IN | globose | 4.5–6.0 |
| A. leucospermum | – | – | – | – | – |

Notes: Arthrinium arctoscopi is closely related to A. obovatum (98.84% similarity in the ITS region, 96.10% in the TEF region, and 94.31% in the TUB region) and A. aquaticum (99.80% similarity in the ITS region). However, A. arctoscopi can be distinguished from A. obovatum by the conidial shape and growth rate; the conidia of A. arctoscopi are globose to subglobose, whereas those of A. obovatum are obvoid or occasionally elongated to ellipsoidal in shape (Wang et al. 2018). In addition, the growth rate of A. arctoscopi (7–9 mm in 7 d at 25°C, PDA) is slower than that of A. obovatum (covering a 90 mm Petri dish in 7 d at 25°C, PDA) (Wang et al. 2018). The conidial shape of A. arctoscopi is also slightly different from that of A. aquaticum (globose to subglobose conidia, 9–11 × 8–10 μm, = 10 × 9 μm, n = 20). Two non-sequenced spe-

mycelia white; sporulation on hyphae after 2 weeks, spores black; pigment absent in medium; odour indistinct. OA: colonies thick, concentrically spreading with aerial mycelium, margin irregular; mycelia creamy pale yellow; sporulation not observed; very dark greyish brown (2.5Y 3/2) pigment diffused from centre into medium; odour indistinct. Colony diameters (in mm after 120 h): 15°C PDA 9, MEA 13–15, OA 11–13; 20°C PDA 18–24, MEA 18–22, OA 14–18; 25°C PDA 5–7, MEA 4–5, OA 7–9.

Additional material examined: Korea: Gangwon-do, Goseong-gun, 38°28′44.0″N, 128°26′18.9″E, isolated from egg masses of Arctoscopus japonicus, 10 Nov. 2016, M.S. Park (KUC21344, KUC21345, KUC21346, and KUC21347).
cies, *A. algicola* and *A. sinensis*, are morphologically similar to *A. arctoscopi*. The longer length and narrower width of *A. algicola* conidia (10.5–15 × 6–8 μm) and lageniform conidiogenous cell of *A. sinensis* distinguish them from *A. arctoscopi* (Table 2).

**Arthrinium fermenti** S.L. Kwon, S. Jang & J.J. Kim, sp. nov.

MycoBank MB834594

(Fig. 5)

Etymology: ‘fermenti’ refers to the yeast-like odour of the cultures.

**Molecular diagnosis:** *Arthrinium fermenti* is distinguished from the phylogenetically most closely related species, *A. pseudospegazzinii*, by unique single nucleotide polymorphisms in the three loci used in this study (Figs. 3S, 4S, 5S): ITS positions 32 (C), 43 (T), 81 (C),
283 (T), 318 (T), 567 (A), and 644 (indel); TEF positions 9 (C), 35 (C), 44 (A), 67 (A), 81–82 (indel), 84 (indel), 87 (C), 92 (G), 93 (A), 114 (G), 126 (C), 133 (T), 134 (G), 140 (T), 154 (G), 170 (C), 171 (T), 172 (T), 178 (indel), 181 (indel), 192 (C), 206 (indel), 208–211 (indel), 213 (T), 239 (G), 243 (T), 252 (A), 264 (C), 288 (G), 305 (C), 311 (C), 322 (indel), 330 (A), 337 (T), 357 (G), 367 (T), 375 (T), 392 (A), and 473 (T); TUB positions 1 (T), 9 (T), 18–22 (indel), 28 (A), 33 (C), 41 (G), 67 (A), 80 (A), 94 (G), 106 (T), 117 (T), 223 (A), 233 (T), 308 (A), 309 (T), 322 (T), 327 (C), 329 (C), 331 (C), 425 (C), and 437 (T).

**Type:** Korea: Jeollanam-do, Haenam-gun, 34°26'07.2"N, 126°28'16.5"E, isolated from seaweed, 23 Apr. 2014, M.S. Park (Herb. KCTC 46903 – holotype preserved in a
metabolically inactive state; KUC21289 = NIBRFGC000501584, SFC20140423-M86 – ex-type cultures).

**Description:** Mycelium of smooth, hyaline, branched, septate, 2.0–4.0 μm diam. Conidiogenous cells aggregated in clusters on hyphae, at first hyaline, becoming pale brown, polyblastic, discrete, erect, ampulliform. Conidia brown, smooth to granular, globose to elongated ellipsoidal, (7.5–)8.0–9.0 × 7.0–8.5 (–9) μm (μ = 8.32 × 7.4 μm, n = 30); lenticular in side view, with equatorial slit, 6.0–7.0 μm wide (μ = 6.6 μm, n = 30).

**Culture:** PDA: colonies thick, concentrically spreading with aerial mycelium, margin irregular; mycelia white to yellow, becoming pinkish to orange after 2 weeks; sporulation on hyphae, spores black; dark reddish brown (5YR 2.5/2) to yellow (2.5Y 8/8) pigment diffused from centre into media; odour strong baker yeast. MEA: colonies low, flat, concentrically spreading, thin, margin irregular; mycelia white; sporulation was not observed; medium reverse with yellow pigment after 2 weeks; odour strong baker’s yeast-like. OA: colonies thick, concentrically spreading with aerial mycelium, margin irregular; mycelia at first white, reverse randomly pale pink to red-grape and pale yellow to brown after 2 weeks; sporulation on hyphae, spores black; dark yellowish brown (10YR 3/4, 3/6) to dark reddish brown (2.5YR 2.5/4) pigment diffused into the medium; odour strong baker’s yeast-like. Colony diameters (in mm after 120 h): 15°C PDA 17, MEA 17–18, OA 13–16; 20°C PDA 27–30, MEA 21–27, OA 15–18; 25°C PDA 21–23, MEA 18–19, OA 14–16.

**Additional material examined:** Korea: Jeollanam-do, Haenam-gun, 34°26′07.2″N, 126°28′16.5″E, isolated from seaweed, 23 Apr. 2014, M.S. Park (KUC21288).

Notes: Arthrinium fermenti is closely related to A. pseudospegazzinii (98.96% similarity in the ITS region, 92.47% in the TEF region, and 95.00% in the TUB region) (Figs. 1, 2). It can be distinguished from the latter by conidial shape and colony colour. The conidia of A. fermenti are globose to elongate-ellipsoid, whereas A. pseudospegazzinii has uniformly globose conidia (Crous & Groenewald, 2013). Moreover, while the colonies of A. pseudospegazzinii were light orange on PDA and dirty white with an olivaceous grey patch on OA and MEA (Crous & Groenewald, 2013), A. fermenti colonies had a yellowish to reddish colour on OA and MEA and a strong yeast odour. Arthrinium globosum (non-sequenced species) has a conidial shape similar to that of A. fermenti – globose to subglobose. However, a lenticular shape in side view was not observed in A. globosum (Table 2).

**Arthrinium koreanum** S.L. Kwon, S. Jang & J.J. Kim, **sp. nov.**

Mycobank MB834596 (Fig. 6)
Arthrinium koreanum has a similar conidia shape to that of the two non-sequenced species, A. globosum and A. sphaerospermum. However, the conidia of the latter two species only have globose to subglobose shape, and lenticular shape is not observed in side view (Table 2).

Arthrinium marinum S.L. Kwon, S. Jang & J.J. Kim, sp. nov.

MycoBank MB834595 (Fig. 7)

Etymology: 'marinum' refers to the marine origin.

Molecular diagnosis: Arthrinium marinum is distinguished from the phylogenetically most closely related species, A. rasikravindrae, by unique single nucleotide polymorphisms in the three loci used in this study (Figs. 3S, 4S, 5S): ITS positions 100% similarity; TEF positions...
191 (T), 253 (C), 256 (A), 319 (A), and 372 (C); TUB positions 2 (T), 15 (A), 20 (G), 30 (C), 69 (G), 111 (indel), 314 (G), 363 (T), 437 (C), and 443 (C).

**Type: Korea:** Jeollanam-do, Suncheon-si, 34°50′46.9″N, 127°31′31.4″E, isolated from seaweed, 23 Apr. 2014, M.S. Park (Herb. KCTC 46905 – holotype preserved in a metabolically inactive state; KUC21328 = NIBRFGC000501583, SFC20140423-M02 – ex-type cultures).

**Description:** Mycelium superficial, composed of smooth, hyaline, branched, septate, 3.5–6.0 μm diam. Hyphae. Conidiogenous cells aggregated in clusters on hyphae or solitary, hyaline, erect, ampulliform. Conidia brown, smooth to granular, globose to elongate ellipsoid in surface view, (9.5–)10–12 (−13) × (7.5–)8.0–10 μm ($\bar{x} = 11.1 \times 9.4 \mu m$, $n = 30$); lenticular in side view, with equatorial slit, 6.0–7.5 μm wide ($\bar{x} = 7.1 \mu m$, $n = 30$).
**Culture**: PDA: colonies thick and dense, concentrically spreading, margin irregular; mycelia white to pale yellow; sporulation was not observed; pale yellow (5Y 8/4) pigment diffused into medium; odour indistinct. MEA: colonies low, flat, concentrically spreading with sparse aerial mycelium, margin circular; mycelia white colored; sporulation on hyphae around centre after 2 weeks, spores black; pigment absent in medium; odour indistinct. OA: colonies thick, concentrically spreading with aerial mycelium, margin circular; mycelia white to pale yellow; sporulation not observed; yellow to pale green (2.5Y 5/6) pigment diffused into medium; odour indistinct. Colony diameters (in mm after 120 h): 15 °C PDA 7–9, MEA 6–12, OA 4–5; 20 °C PDA 16–17, MEA 14–21, OA 7–9; 25 °C PDA 35–47, MEA 32–35, and OA 28–32.

Additional material examined: **Korea**: Jeollanam-do, Suncheon-si, 34°50′46.9″N, 127°31′31.4″E, isolated from seaweed, 23 Apr. 2014, **M.S. Park** (KUC21353, KUC21354, KUC21355, and KUC21356).

**Notes**: Although *Arthrinium marinum* and *A. rasikravindrae* were not distinguished on ITS alone (100% similarity in the ITS region), these species formed two distinct clades based on the combined analysis of the ITS, TUB, and TEF regions (99.08% in the TEF region and 97.97% in the TUB region) (Figs. 1, 2). They can also be distinguished by their growth rates: *A. marinum* (16–17 mm in 5 d on PDA at 20 °C) had a slower growth rate than *A. rasikravindrae* KUC21327 (34–39 mm in 5 d on PDA at 20 °C).

Non-sequenced species, *Arthrinium algicola*, has a very similar conidia shape to that of *A. marinum*. However, they are distinguished by the conidia size; 10.5–15 × 6–8 μm in *A. algicola* and (9.5)–10–12–(13) × (7.5)–8–10 μm in *A. marinum* (Table 2).

*Arthrinium pusillispermum* S.L. Kwon, S. Jang & J.J. Kim, sp. nov.

MycoBank MB834597

(Fig. 8)

**Etymology**: ‘pusillus’, tiny and ‘spermum’ spores.

Molecular diagnosis: *Arthrinium pusillispermum* is distinguished from the phylogenetically most closely related species, *A. gutiae*, by unique single nucleotide polymorphisms in the three loci used in this study (Figs. 3S, 4S, 5S): ITS positions 43 (C), 260 (T), and 546 (T); TEF positions 1–17 (indel), 26–38 (indel), 43–46 (indel), 64–69 (indel), 76–82 (indel), 84–96 (indel), 112–115 (indel), 125–131 (indel), 137–141 (indel), 151–172 (indel), 173 (C), 174 (A), 175 (G), 178 (G), 180 (T), 192 (T), 193 (indel), 194 (G), 209 (A), 213 (indel), 228 (A), 230 (C), 243 (C), 251 (C), 252 (A), 256 (A), 260 (A), 261 (A), 264 (T), 268 (G), 269 (T), 273–276 (indel), 287–289 (indel), 293 (A), 294 (G), 308 (A), 310 (G), 313 (C), 314 (indel), 315 (C), 321 (T), 325 (indel), 327 (indel), 328 (A), 332 (indel), 337 (T), 356 (C), 358 (A), 360 (T), 364 (C), 374 (A), 395 (C), and 473 (T); TUB position 38 (C), 75 (T), 89 (G), 144 (A), and 498–506 (indel).

**Type**: Korea: Chungcheongnam-do, Taean-gun, 36°50′14.3″N, 126°11′04.7″E, isolated from seaweed, 19 Mar. 2016, **S. Jang** (Herb. KCTC 46906 – holotype preserved in a metabolically inactive state; KUC21321 = NIBRFGC000501585 – ex-type culture).

**Description**: Mycelium consisting of smooth, hyaline, branched, septate, 1.5–4.5 μm diam. Conidiogenous cells aggregated in clusters on hyphae, hyaline, cylindrical. Conidia brown, smooth to granular, globose to subglobose in surface view, 4.0–6.0 (–6.5) × (3.0–)3.5–5.0 (–5.5) μm (x = 5.1 × 4.2 μm, n = 30); lenticular in side view, with equatorial slit, 3.5–4.5 μm wide (x = 4.1 μm, n = 30), elongated cell present.

**Culture**: PDA: colonies thick around centre, concentrically spreading with aerial mycelium, margin circular; mycelia white, pale yellow to grey; sporulation was not observed; greenish black (10GY 2.5/1) pigment diffused in medium; odour indistinct. MEA: colonies abundant, flat, concentrically spreading with sparse aerial mycelium, margin irregular; mycelia white to gray colored; sporulation was not observed; pigment absent in medium; odour indistinct. OA: colonies thick, concentrically spreading with aerial mycelium, margin irregular; mycelia white to pale brown and grey to dark grey; sporulation on hyphae around the centre after 2 weeks, spores black; greenish black (10Y 2.5/1) to very dark greenish grey (10Y 3/1) pigment diffused in medium; odour indistinct. Colony diameters (in mm after 120 h): 15 °C PDA 19–25, MEA 10–12; 20 °C PDA 25–39, MEA 19–25, OA 22–24; 25 °C PDA 9–15, MEA 6–18, and OA 6–20.

Additional material examined: **Korea**: Chungcheongnam-do, Taean-gun, 36°50′14.3″N, 126°11′04.7″E, isolated from seaweed 19 Mar. 2016, **S. Jang** (KUC21357).

**Notes**: *Arthrinium pusillispermum* is closely related to *A. gutiae* (99.44% similarity in the ITS region, 88.52% in the TEF region, and 98.98% in the TUB region) (Figs. 1, 2). *Arthrinium pusillispermum* is distinguished from *A. gutiae* by the shape of the conidiogenous cells and the substrate: *A. pusillispermum* has cylindrical conidiogenous cells and was isolated from seaweed, whereas *A. gutiae* has lageniform conidiogenous cells and was isolated from the gut of grasshoppers (Crous et al. 2015). *Arthrinium pusillispermum* can be distinguished from the 22 non-sequenced species by its small conidia size (Table 2).

*Arthrinium sargassi* S.L. Kwon, S. Jang & J.J. Kim, sp. nov.

MycoBank MB834598
Etymology: ‘sargassi’ refers to the genus name of Sargassum sp., the substrate of the type material.

Molecular diagnosis: Arthrinium sargassi is distinguished from the phylogenetically related species, A. hydei, by unique single nucleotide polymorphisms in the three loci used in this study (Figs. 3S, 4S, 5S): ITS positions 31 (C), 47 (indel), 91 (C), 95 (indel), 309 (T), and 644 (indel); TEF positions 15 (C), 27 (C), 30 (T), 37 (C), 46 (T), 47 (indel), 63 (indel), 64 (C), 66 (T), 67 (A), 92 (C), 93 (A), 95 (G), 140 (G), 152 (C), 153 (A), 155 (G), 160 (T), 193 (T), 222 (C), 224 (A), 225 (C), 253 (C), 254 (C), 262 (C), 265 (T), 293 (A), 328 (A), 336 (A), 358 (T), 367 (A), 371 (T), 374 (C), 376 (A), 386 (C), 392 (A), and 449 (C); TUB positions 10 (C), 18 (C), 22 (T), 23 (G), 30 (T), 45
(T), 47 (A), 52 (A), 69 (A), 70 (C), 80 (G), 106 (T), 133 (A), 145 (A), 225 (A), 230 (G), 380 (T), 416 (T), and 437 (T).

**Type: Korea:** Jeju-do, 33°23′39.2″N, 126°14′23.0″E, isolated from *Sargassum fulvellum*, 10 Jan. 2015, S. Jang (Herb. KCTC 46901 – holotype preserved in a metabolically in-active state; KUC21228 = NIBRFGC000501578 – ex-type culture).

**Description:** Mycelium consisting of smooth, hyaline, branched, septate, 2.0–5.0 μm diam. Conidiogenous cells aggregated in clusters on hyphae or solitary, at first hyaline, becoming pale brown, basauxic, polyblastic, sympodial, erect, cylindrical. Conidia brown, smooth to granular, globose to subglobose in surface view, (8.5–)9.5–11 (~ 11.5) × (8.0–)8.5–10 (~ 11) μm (X = 10.4 × 9.4 μm, n = 30); lenticular in side view, with...
equatorial slit, 5.5–7.5 μm wide (X = 6.5 μm, n = 30), elongated cell present.

**Culture:** PDA: colonies thick, flat, concentrically spreading with aerial mycelium, margin circular; mycelia white to grey, reverse sparsely pale yellow; sporulation on hyphae and in medium after 2 weeks, randomly dense, spores black; yellow (10YR 8/8) pigment diffused in medium from centre, sometimes remaining as dark grey spots; odour indistinct. MEA: colonies slightly thick, flat, concentrically spreading with aerial mycelium, margin circular; mycelia white coloured; sporulation on hyphae and in medium after 2 weeks, randomly dense, spores black; pigment absent, sometimes remaining dark grey spots in medium; odour indistinct. OA: colonies thick and dense, flat, concentrically spreading with aerial mycelium, margin circular; mycelia white, reverse usually yellow to green from the centre, sometimes becoming pinkish after 2 weeks; sporulation on hyphae, randomly dense after 2 weeks, sporoes black; yellow (2.5Y 7/8) pigment diffused in medium; odour indistinct. Colony diameters (in mm after 120 h): 15 °C PDA 10–12, MEA 15–23, OA 14–15; 20 °C PDA 21–26, MEA 20–27, OA 25–27; 25 °C PDA 29–32, MEA 26–28, and OA 30–34.

**Additional material examined:** **Korea:** Jeju-do, 33°23′39.2″N, 126°14′23.0″E, isolated from Sargassum fulvellum, 10 Jan. 2015, S. Jang (KUC21232, KUC21284, and KUC21287).

**Notes:** Arthrinium sargassi has morphological characteristics similar to those of other species in clade B. It can be distinguished from A. aureum (globose to ellipsoid conidia, 10–30 × 10–15 μm) and A. hydei (globose conidia, 17–19 μm diam) in the much smaller conidia, (8.5–9.5) × (8.0–8.5–10–11 μm) (X = 10.4 × 9.4 μm, n = 30) (Calvo 1980; Crous & Groenewald 2013). Arthrinium rasikravindrae KUC21327 (34–39 mm in 5 d on PDA at 20 °C) and A. marinum (16–17 mm in 5 d on PDA at 20 °C) can be distinguished from A. sargassi (21–26 mm in 5 d on PDA at 20 °C) by their growth rate. Unfortunately, there are no data regarding the growth rate of A. chinense, but it can be clearly separated from A. sargassi based on the phylogenetic analysis (Figs. 1, 2). Arthrinium sargassi is morphologically similar to A. sinensis, a non-sequenced species. However, the shape of conidiogenous cell differs between them; lageniform in A. sinensis and cylindrical in A. sargassi (Table 2).

**Arthrinium taeanense** S.L. Kwon, S. Jang & J.J. Kim, sp. nov.

Mycobank MB8834599

(Fig. 10)

**Etymology:** taeanense refers to the type locality.

**Molecular diagnosis:** Arthrinium taeanense is distinguished from the phylogenetically most closely related species, A. gutiae, by unique single nucleotide polymorphisms in the three loci used in this study (Figs. 3S, 4S, 5S): ITS positions 22 (A), 32 (indel), 43 (G), 48 (C), 109 (indel), 113 (T), 121 (T), 129–146 (indel), 149–156 (indel), 189–192 (indel), 202–211 (indel), 213 (indel), 221 (T), 227–228 (indel), 248–250 (indel), 253 (C), 257 (T), 263 (A), 283 (G), 300 (T), 308 (C), 535 (C), 536 (G), 546 (T), 591 (A), 592 (T), and 593 (T); TEF positions 173 (T), 174 (C), 175 (A), 176 (C), 179 (C), 180 (T), 189 (G), 194 (G), 200 (indel), 209 (A), 213 (indel), 214 (C), 226 (A), 228 (A), 229 (A), 230 (C), 251 (C), 252 (T), 253 (T), 260 (A), 263 (C), 264 (T), 265 (A), 266 (T), 269 (T), 270 (T), 272 (G), 273–275 (indel), 278 (T), 280 (indel), 281 (A), 287 (G), 289 (C), 293 (A), 302 (indel), 304 (indel), 307 (G), 308 (G), 309 (indel), 310 (A), 313 (A), 314 (indel), 318 (G), 334 (G), 337 (T), 356 (A), 357 (G), 358 (A), 371 (T), 374 (A), 375 (G), 376 (G), 378 (C), 395 (C), 404 (C), 467 (T), and 600 (C); TUB positions 2 (T), 3 (C), 7 (C), 10 (C), 11–12 (indel), 16 (G), 17 (T), 19 (A), 20 (C), 21 (A), 22 (T), 23 (C), 25 (C), 26 (G), 28 (G), 29 (A), 33 (C), 34 (C), 35 (T), 36 (C), 38 (C), 41 (T), 44 (A), 46 (G), 53 (A), 54 (T), 68 (T), 69 (C), 71 (A), 72 (A), 73 (T), 74 (A), 75 (T), 78 (T), 80 (G), 81 (G), 85 (G), 87 (G), 89 (G), 95 (C), 108 (G), 111 (G), 114 (A), 129 (T), 138 (C), 140 (T), 143 (T), 146 (T), 158 (C), 170 (C), 176 (C), 184 (A), 198 (C), 205 (A), 207 (C), 211–212 (indel), 214–216 (indel), 231 (G), 308 (C), 309 (C), 312 (C), 313 (T), 319 (T), 324 (C), 326 (G), 327 (C), 328 (C), 329 (T), 344 (T), 347 (T), 353 (C), 392 (A), 395 (T), 410 (C), 413 (G), 416 (C), 425 (C), 428 (T), 434 (C), 437 (G), 455 (T), 476 (T), 479 (C), and 485 (C).

**Type:** Korea: Chungcheongnam-do, Taean-gun, 36°50′14.3″N, 126°11′04.7″E. isolated from Seaweed, 19 Mar. 2016, S. Jang (Herb. KCTC 46910 – holotype preserved in a metabolically inactive state; KUC21322 = NIBRFGC000501589 – ex-type culture).

**Description:** Mycelium consisting of smooth, hyaline, branched, septate, 2.0–4.5 μm diam. Conidiogenous cells aggregated in clusters on hyphae, hyaline, cylindrical. Conidia brown, smooth to granular, globose to elongate ellipsoid in surface view, (5.0–)5.5–6.5 (– 7.0) × 4.0–5.5 (– 6.0) μm (X = 6 × 4.7 μm, n = 30); lenticular in side view, with an equatorial slit, 4.0–5.0 μm wide (X = 4.7 μm, n = 30), elongated cell observed.

**Culture:** PDA, colonies thick, concentrically spreading with aerial mycelium, margin circular; mycelia white to yellow, gray and partially pale orange colored; sporulation was not observed; pale yellow (5Y 8/3) pigment to yellow (2.5Y 8/8) pigment diffused in medium after 2 weeks; odour indistinct. MEA, colonies thick, flat, concentrically spreading with aerial mycelium, margin circular; mycelia white to yellowish grey colored; sporulation was not observed; pigment absent in medium; odour indistinct. OA, colonies very thick,
concentrally spreading with aerial mycelium, margin circular; mycelia white to yellow and orange to brown colored; sporulation was not observed; yellowish brown (10YR 5/8) pigment diffused in media after 2 weeks; odour indistinct. Colony diameters (in mm after 120 h): 15 °C PDA 7–15, MEA 10–20, OA 10–11; 20 °C PDA 28–36, MEA 24–32, OA 21–24; 25 °C PDA 36–39, MEA 34–35, and OA 39–41.

Additional material examined: Korea: Chungcheongnam-do, Tae-an-gun, 36°50′14.3″N, 126°11′04.7″E, isolated from seaweed, 19 Mar. 2016, S. Jang (KUC21358, KUC21359).

Notes: Arthrinium taeanense is most closely related to A. pusillispermum (95.30% similarity in the ITS region, 80.84% in the TEF region, and 79.30% in the TUB region) and A. gutiae (95.30% similarity in the ITS region, 85.19% in the TEF region, and 78.3% in the TUB region).
(Fig. 1). There were no noticeable morphological characters that helped separate these species, but the long stem branches clearly indicate that they represent different, phylogenetically well-separated taxa. *Arthrinium taeanense* can be distinguished from the 22 non-sequenced species by its small conidia size (Table 2).

**DISCUSSION**

A total of 14 *Arthrinium* species associated with marine environments in Korea was identified based on morphological and molecular phylogenetic analyses. Five species, *A. arundinis*, *A. marii*, *A. rasikravindrae*, *A. sacchari*, and *A. saccharicola*, had already been reported from marine environments (Hong et al. 2015; Park et al. 2018), whereas *A. piptatheri* was reported here for the first time from this habitat. The newly recognized taxa represented six species isolated from macroalgae (*A. agari*, *A. fermenti*, *A. marinum*, *A. pusillispernum*, *A. sargassi*, and *A. taeanense*) and two extracted from the egg masses of saffin sandfish (*A. arctoscopi* and *A. koreanum*). To date, the majority of the described *Arthrinium* species have been isolated from various terrestrial habitats (Tsukamoto et al. 2006; Kim et al. 2011; Crous & Groenewald, 2013), whereas only eight *Arthrinium* species have been reported from marine environments: *A. algicola*, *A. arundinis*, *A. hispanicum*, *A. marii*, *A. phaeospernum*, *A. rasikravindrae*, *A. sacchari*, and *A. taeanense* (Miao et al. 2006; Jones et al. 2009; Crous & Groenewald 2013; Hong et al. 2015; Larrondo 1992; Li et al. 2017; Park et al. 2018; Pintos et al. 2019).

As mentioned, conidial shape, conidiophores, and presence or absence of sterile cells and setae were previously used for the infrageneric classification and delimitation of species (Schmidt & Kunze 1817; Hughes 1953; Minter 1985). However, because these microscopic features often overlap between taxa, it is difficult to solely rely on them to distinguish species. Therefore, the combined use of molecular and morphological characters, in combination with the physiological features of the cultures, is required to identify species in *Arthrinium*. For example, the newly recognized species, *A. marinum*, *A. pusillispernum*, and *A. taeanense*, cannot be distinguished from their close relatives based on morphology alone; however, the three species could be distinguished by differences in their growth rate and by the molecular data.

*Arthrinium* species can be divided into two groups based on conidial shape: one group with an irregular conidial shape, similar to a cashew-nut (*A. kamischaticum*) or a polygon (*A. puccinioides*), and the other with globose to ellipsoid conidia. This corresponds to the conidial shape of other *Arthrinium* species derived from marine environments (Larrondo 1992; Crous and Groenewald 2013; Singh et al. 2013). Among the species with ellipsoid conidia, those from marine environments generally have more elongated conidia than those from terrestrial environments (Table 2). There are a number of *Arthrinium* species described only from their sexual morph (e.g., *A. balearicum*, *A. garethjonesii*, *A. longistromum*, *A. neosubglobosa*, *A. subglobosa*) (Sendnayake et al. 2015; Dai et al. 2016; Dai et al. 2017; Pintos et al. 2019). Unfortunately, no sexual morph is known in any of the marine species. This further increases the difficulty of identifying *Arthrinium* species through morphological features alone.

DNA sequencing data available for *Arthrinium* species has been steadily increasing in recent years (Crous and Groenewald 2013; Wang et al. 2018; Pintos et al. 2019). Currently 84 species of *Arthrinium* are recognized; of these, sequence information on the ITS is available for 62 species, TUB for 51, and TEF for 45 species. This has contributed to an increase in newly recognized species and aids in their accurate and rapid identification (Wang et al. 2018; Pintos et al. 2019). ITS by itself is limited in its ability to identify species within *Arthrinium*. The use of TUB, TEF, and multigene sequence data (ITS, TUB, and TEF) has increased the accurate identification and phylogenetic relationships in *Arthrinium*. This study generated 67 sequence datasets for three gene regions (ITS, TUB, and TEF), which will also contribute to furthering the study of the genus *Arthrinium*.

According to our previous studies on marine *Arthrinium* species, the 14 identified in this study can be expected to have high biological activity. However, it is not clear whether they are active in the actual marine environment and what the ecological role of *Arthrinium* species is. We expect to better understand their role in the environment through various studies of *Arthrinium* species in the future, including the discovery of further novel species and an exploration of their biological properties.

**CONCLUSIONS**

Our study underlines the notion that the diversity of *Arthrinium* species is still poorly known. More than half of the *Arthrinium* species isolated from a limited marine environment resulted to be new to science. According to our results, many more novel taxa are to be expected from marine environments around the world. Further studies in other environments are needed to assess the distribution of these species. Our results also show that a polyphasic approach to the taxonomy of *Arthrinium*, integrating molecular phylogeny of ITS and protein-coding markers, conidial features and culture characteristics are the most reliable approach to delimit and recognize species in this genus.
Abbreviations
Bi: Bayesian tree inference; bp: base pair; BS: Bootstrap support; diam: diameter; DNA: Deoxynucleobasic acid; Herb: Herbarium; ITS: Internal transcribed spacer; KCTC: Korean Collection for Type Culture; KUC: Korea University Fungus Collection; MEA: Malt extract agar; ML: Maximum likelihood; OA: oatmeal agar; PCR: Polymerase chain reaction; PDA: Potato dextrose agar; PP: Posterior probabilities; ROS: Reactive oxygen species; SEM: Scanning electron microscope; SFC: Seoul National University Fungus Collection; TEF: Translation elongation factor 1-alpha; TUB: β-tubulin

Supplementary Information
The online version contains supplementary material available at https://doi.org/10.1186/s43008-021-00065-z.

Additional file 1: Fig. S1. ML tree based on the TEF region. The numbers at the nodes indicate ML bootstrap support (BS) > 75% and Bayesian posterior probabilities (PP) > 0.75 as BS/PP. The thickened branches indicate support greater than 85% for BS and 0.95 for PP. A hyphen (“-”) indicates values of BS < 70% or PP < 0.75. Ex-holotype strains are indicated with asterisks (“*”). The fungal cultures examined in this study are shown in bold. Red boxes indicate the novel species. The numbers in the brackets indicate strain number. The scale bar indicates the nucleotide substitutions per position. Fig. S2. ML tree based on the TUB region. The numbers at the nodes indicate ML bootstrap support (BS) > 75% and Bayesian posterior probabilities (PP) > 0.75 as BS/PP. The thickened branches indicate support greater than 85% for BS and 0.95 for PP. A hyphen (“-”) indicates values of BS < 70% or PP < 0.75. Ex-holotype strains are indicated with asterisks (“*”). The fungal cultures examined in this study are shown in bold. Red boxes indicate the novel species. The numbers in the brackets indicate strain number. The scale bar indicates the nucleotide substitutions per position. Fig. S3. Sequence alignments of ITS regions of eight novel Arthrinium. Fig. S4. Sequence alignments of TEF regions of eight novel Arthrinium. Fig. S5. Sequence alignments of TUB regions of eight novel Arthrinium.

Additional file 2: Table S1. Sequence information of Arthrinium species. Newly established species in this study are shown in bold.

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Adherence to national and international regulations
Not applicable.

Authors’ contributions
MSP, SJ, YML, JH, HL, and YJ collected samples. SLK, MSP, SJ, YMH and JH isolated cultures and performed DNA isolation and PCR amplification. SLK and MSP analyzed data. SLK and MSP wrote the original draft, JP, CK, GK, and YJH reviewed and edited the draft and contributed to the discussion. YWL and JK supervised this research. All authors read and approved the manuscript.

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Availability of data and materials
All data generated or analyzed during this study are included in this published article.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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