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Rapid Communication

First record of the invasive ascidian *Microcosmus squamiger* Michaelsen, 1927 (Ascidiacea: Pyuridae) in Jeju Island, South Korea

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Abstract

Ascidians are sessile marine invertebrates and many are invasive. One notorious example is the solitary species *Microcosmus squamiger*, which has spread from a hypothesized native region in southeastern Australia to temperate waters worldwide and is considered a global marine invader. This study is the first report of *M. squamiger* from Jeju Island, South Korea. We collected specimens (N = 20) from Unjin and Wimi harbors on Jeju Island and dissected them to characterise their siphons, pharynx, and dorsal tubercle using stereomicroscopy and scanning electron microscopy. The shape of the dorsal tubercle (the peritubercular area filled with two spiral coils) and the length (15–20 µm) and shape (roof tile or fingernail) of the siphon spine were consistent with previous identifications of the species. Additionally, molecular verification was performed by extracting genomic DNA from four specimens, two each from Unjin and Wimi harbors. By sequencing a section of the mitochondrial cytochrome oxidase subunit I gene and analysing the generated sequences using a maximum likelihood-based phylogenetic tree, we compared to individuals previously identified as *M. squamiger* and confirmed the existence of two clades. Therefore, the presence of *M. squamiger* was verified by morphological and phylogenetic identification. Although *M. squamiger* is currently distributed globally (except in Antarctica), records from India (2006), Okinawa, and Japan (2007 and 2014) suggest that it was introduced into Asia in the last decade. Though no economic or biological damage caused by this species has been reported in South Korea, the high density of *M. squamiger* (50–60 individuals/rope-meter) is a potential hazard to other native species via fouling and competition for food and space. Therefore, further research is needed to determine the possible vectors and to continue to monitor its possible spread.

Key words: non-indigenous species, cytochrome oxidase I, tunicate, biofouling, siphonal spines

Introduction

Contemporary climate change and human activities can lead to rapid fluctuations in the distribution of coastal marine species on a global scale (Dukes et al. 1999; Harley et al. 2006; Occhipinti-Ambrogi 2007). The southern coast of Jeju Island (South Korea) is the first area to be directly affected by the high temperature and salinity of the Tsushima Warm Current as it flows from the tropical south along the Korean Peninsula. Recently, this area has been exposed to an increased volume of the

Tsushima Warm Current due to changes in oceanic circulation, which has been strengthened by climate change (Pang et al. 1996; Zhang et al. 2000; Rebstock and Kang 2003). Consequently, new records of various tropical/subtropical marine species have been continuously reported on Jeju Island (Kim et al. 2013; Denis et al. 2014; Lee et al. 2015; Park et al. 2017; Kwun and Jung 2018; Reimer et al. 2018; Yang et al. 2018; Kwun 2020; Song et al. 2020; Joh 2021). These reports, providing examples of the transition from subtropical to tropical zones, are drawing the attention of researchers (Jung et al. 2013; Kang et al. 2011; Kim et al. 2015; Hyun et al. 2020; Lee et al. 2021).

Ascidians are sessile marine invertebrates that are often invasive (Lambert 2001; Aldred and Clare 2014; Zhan et al. 2015; Chan and Briski 2017). They can withstand a wide range of variation in salinity and water temperature and can tolerate pollution as well (Lambert 2005; Nagar and Shenkar 2016; Rocha et al. 2017). Owing to a short larval period, the natural dispersal ability of ascidians is limited; however, ascidians migrate worldwide through anthropogenic transport (Carlton and Geller 1993; Monniot and Monniot 1994; Zhan et al. 2015). Once settled successfully, they can outgrow indigenous species through their ability to compete for food and space (Yamaguchi 1975; Carver et al. 2003; Zhan et al. 2015). In the past decade, five new ascidian species have been reported from natural substrates on Jeju Island (Seo and Rho 2014; Seo and Rho 2015; Seo and Rho 2016; Seo 2021); however, *Microcosmus squamiger* Michaelsen, 1927 (Ascidiacea: Pyuridae), the subject of this report, was discovered on an artificial structure.

Microcosmus squamiger is considered a widespread marine invader as it spreads throughout the temperate waters worldwide, altering the biota of the invaded region (Lambert and Lambert 1998, 2003; Turon et al. 2007; Rius et al. 2009). *Microcosmus squamiger* is assumed to have originated in southeastern Australia (Kott 1985; Monniot et al. 2001; Rius et al. 2012), but has spread to the west coast of North America (Lambert and Lambert 1998, 2003), South Africa (Monniot et al. 2001), the east coast of Africa (Monniot 2002), the southern coast of India (Abdul and Sivakumar 2007), the Atlantic Iberian Peninsula coast, and the Mediterranean Sea (Turon et al. 2007). Although the route of its invasion is unknown, this species is reported to dominate a native tunicate, *Styela canopus*, in southern California (Lowe 2002) and is an economic threat to oyster farms due to its fouling of both oysters and lines (Rodriguez and Ibarra-Obando 2008). We consider this species a potential threat to both the local biota and the economy. The objective of our study was to perform morphological and phylogenetic analyses to identify and confirm the presence of *M. squamiger*, a worldwide invasive species, on Jeju Island, South Korea.

Materials and methods

Specimen collection and morphological analysis

Jeju is a volcanic island located at the southernmost tip of the Korean Peninsula in the East China Sea (33°29′20.30″N; 126°29′53.90″E). Specimens

of *M. squamiger* were collected at Unjin (33°12′29.71″N; 126°15′24.00″E) and Wimi (33°16′10.98″N; 126°39′34.50″E) harbor in Jeju Island, South Korea in February 22, 2022, and ten specimens were collected from the ropes of floating docks in each harbor. The specimens were photographed with a camera (TG-6, Olympus, Japan) at the collection site and transported to the laboratory in absolute ethanol (99.5%) for morphological identification and DNA extraction. Voucher specimens (MABIK IV00172391, IV00172392) were deposited at the National Marine Biodiversity Institute of Korea (Seocheon, Korea). The morphological characteristics (Kott 1985; Monniot et al. 2001), including the siphons, pharynx, and dorsal tubercle, of each specimen (N = 5 from Unjin harbor and N = 5 from Wimi harbor) were examined under a stereomicroscope (Leica M205C, Germany) equipped with a digital microscope camera (Leica DFC450C, Germany), which was used to photograph the dorsal nodule. Photographs of the siphon spines were obtained using a scanning electron microscope (SEM, HITACHI SU3500, Japan). Specimens were stained with a Mayer's hematoxylin solution (Sigma-Aldrich, MO, USA).

DNA extraction and sequence analysis

The genomic DNA of *M. squamiger* (N = 2 from Unjin harbor; N = 2 from Wimi harbor) was extracted using a Qiagen DNeasy Blood & Tissue Kit (Hilden, Germany) according to the manufacturer's protocol. The mitochondrial cytochrome oxidase subunit I (COI) gene was partially amplified by polymerase chain reaction (PCR) using the universal primers LCO1490 and HCO2198 (Folmer et al. 1994). The PCR mix consisted of 0.2 µl of PCRBIO HS Taq DNA Polymerase (5 U/µl), 4 µl of 5X PCRBIO Reaction Buffer (PCR Biosystems Ltd, London, UK), 1 µl of forward and reverse primers each (10 pmol), 1 µl of purified genomic DNA (10–30 ng), and 12.8 µl of sterile distilled water. The reaction conditions were as follows: initial denaturation at 95 °C for 5 min, 40 cycles of denaturation at 95 °C for 30 s, annealing at 50 °C for 30 s, extension at 72 °C for 7 min, and final extension at 72 °C for 7 min. The PCR product was observed as a single band on agarose gel (1%) and the nucleotide sequence was obtained by capillary sequencing (G&C Bio Co, Ltd, Daejeon, South Korea).

The forward and reverse sequence reconstruction was performed using Geneious Prime 2021.2.2 (Biomatters Ltd, Auckland, New Zealand). It was followed by alignment with available sequences in NCBI using the standard nucleotide Basic Local Alignment Search Tool (BLAST) and cross checked by the Barcode of Life Data System (BOLD) identification engine (Ratnasingham and Hebert 2007). The reference sequence of NCBI GenBank (https://www.ncbi.nlm.nih.gov/) was used to create a phylogenetic dataset with 33 sequences of *M. squamiger* from introduced and purportedly native locations (including the 4 from this study) and 4 sequences of *Microcosmus polymorphus* (as outgroup; Rius et al. 2008) (Table 1). A model test was

Table 1. Scientific name, specimen location, GenBank Accession numbers, reference of sequences, and clade used for comparison in the maximum likelihood phylogenetic analysis based on COI genetic sequences.

Scientific name	Specimen location	Accession number	Reference	Clade
Microcosmus squamiger	South Korea	ON060763	This study	A
Microcosmus squamiger	South Korea	ON060764		A
Microcosmus squamiger	South Korea	ON060765		A
Microcosmus squamiger	South Korea	ON060766		B
Microcosmus squamiger	South Africa	EU486173	Rius et al. 2008	A
Microcosmus squamiger	Australia	EU486208		B
Microcosmus squamiger	Australia	EU486209		B
Microcosmus squamiger	Australia	EU486210		B
Microcosmus squamiger	Portugal	EU486240		A
Microcosmus squamiger	Portugal	EU486241		A
Microcosmus squamiger	Portugal	EU486244		A
Microcosmus squamiger		EU486306		A
Microcosmus squamiger	Spain	FJ528602	Pérez-Portela et al. 2009	A
Microcosmus squamiger	Spain	FJ528603		A
Microcosmus squamiger	Spain	JQ815436	Pineda et al. 2012	A
Microcosmus squamiger	South Africa	JQ815437		A
Microcosmus squamiger	South Africa	JQ815438		A
Microcosmus squamiger	Spain	JQ815439		A
Microcosmus squamiger	Spain	KF309550	López-Legentil et al. 2015	A
Microcosmus squamiger	Spain	KF309552		A
Microcosmus squamiger	Spain	KF309595		A
Microcosmus squamiger	Spain	KF309600		A
Microcosmus squamiger	India	KF414699	Unpublished from GeneBank	A
Microcosmus squamiger	India	KF414703		A
Microcosmus squamiger	India	KF414707		A
Microcosmus squamiger	Spain	MN185354		A
Microcosmus squamiger	USA	MW872266		A
Microcosmus squamiger	USA	MW872266		A
Microcosmus squamiger	South Africa	MZ882314	Holman et al. 2022	A
Microcosmus squamiger	South Africa	MZ882315		A
Microcosmus squamiger	South Africa	MZ882316		A
Microcosmus squamiger	South Africa	MZ882317		B
Microcosmus squamiger	South Africa	MZ882334		B
Microcosmus polymorphus	Spain	EU486430	Rius et al. 2008	
Microcosmus polymorphus	Italy	OM912472	Unpublished from GeneBank	
Microcosmus polymorphus	Italy	OM912473		
Microcosmus polymorphus	Italy	OM912474		

performed using the PAUP* plugin in Geneious Pime (Swofford 2002) to estimate the optimal model for the phylogenetic tree construction. Maximum likelihood (ML) analysis was conducted using the PhyML plugin in Geneious Pime based on the GTR+I model with 1,000 bootstrap replicates (Guindon et al. 2010). Bootstrap support values above 50% are shown. All sequences obtained in this study were submitted to GenBank (Table 1).

Results

Morphological species identification

In each harbor, the *M. squamiger* formed a high density on the rope (50–60 individuals/rope-meter) (Supplementary material Figure S1a), and five specimens collected from each harbor were used for morphological identification according to the description of previous studies (Kott 1985;

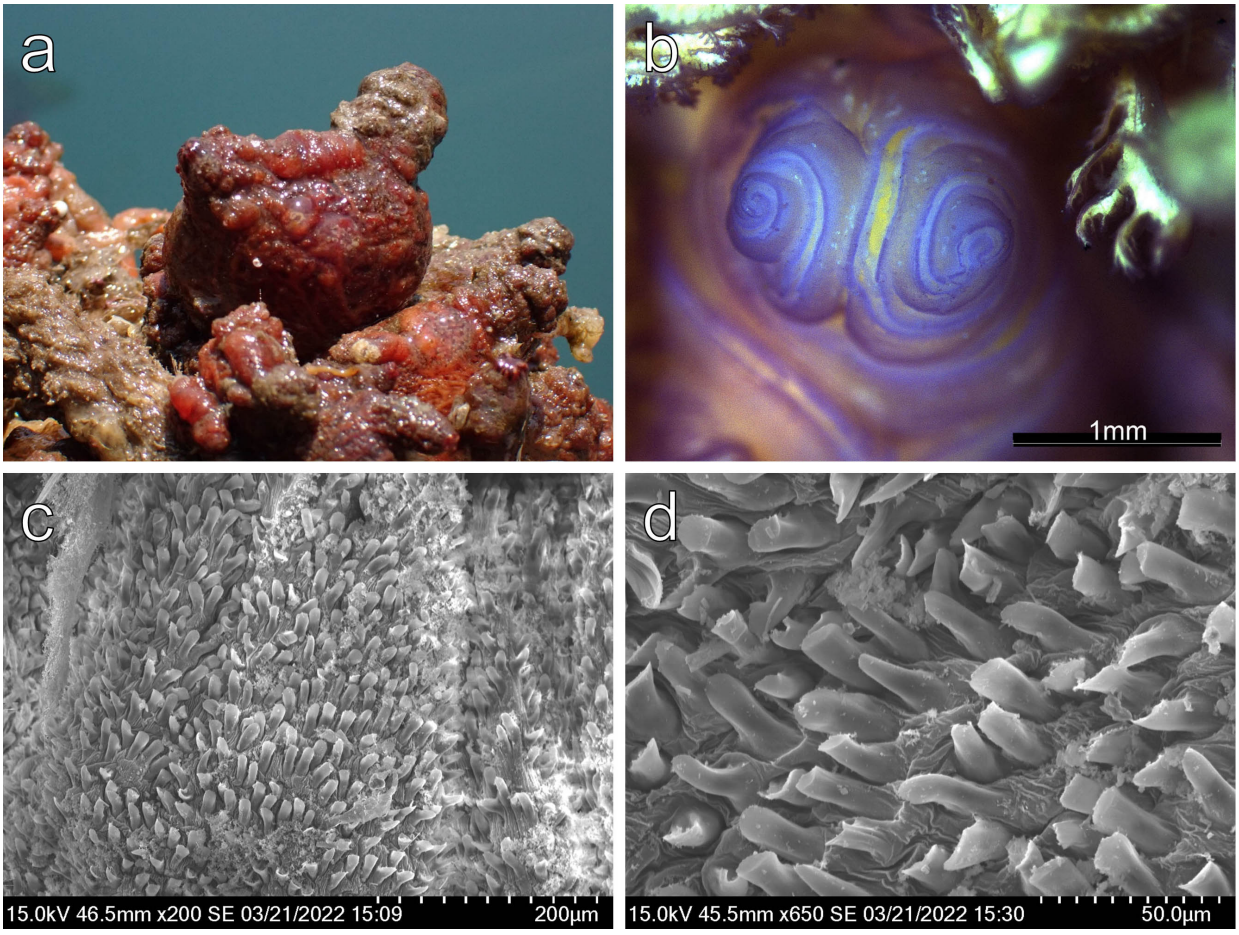

Figure 1. *Microcosmus squamiger* collected from a floating dock (a). Dorsal tubercle in the peritubercular area taken with stereomicroscope (b), and spines in the siphon taken with a scanning electron microscope (c–d). Photographs by Chang-Ho Yi.

Monniot et al. 2001). The body of the specimens, reaching 3 to 5 cm in diameter, was globular with well-separated siphons (Figure 1a). Their fairly tough leathery tunic was colored reddish-brown, usually with other organisms attached to the surface (Figure S1a). The internal tunic was purplish in color and softer in living specimens (Figure S1b). The siphons of the dissected specimens showed a white or yellow stripe extending to approximately 1/2 of the total length. The siphon spines were relatively short (15–20 µm), and the overall shape resembled a roof tile or fingernail with a serrated rim on the edge (Figure 1c, d). The dorsal tubercle protruded significantly and filled the peritubercular area with the opening forming two spiral coil loops in opposite directions (Figure 1b). The pharynx had 6–10 folds on each side and had straight-shaped stigmata (Figure S1c, d). The endostyle was wide, and the button-shaped papillae were irregularly distributed (Figure S1e). The digestive tract was located on the left side, and a digestive gland covered the stomach. The gonads were on both sides of the body wall, and the left gonad extended halfway through the intestine (Figure S1f).

Phylogenetic analysis

The amplified nucleotide sequences (655–657 bp length) were obtained from four specimens, two collected from Unjin (ON060763 and ON060764)

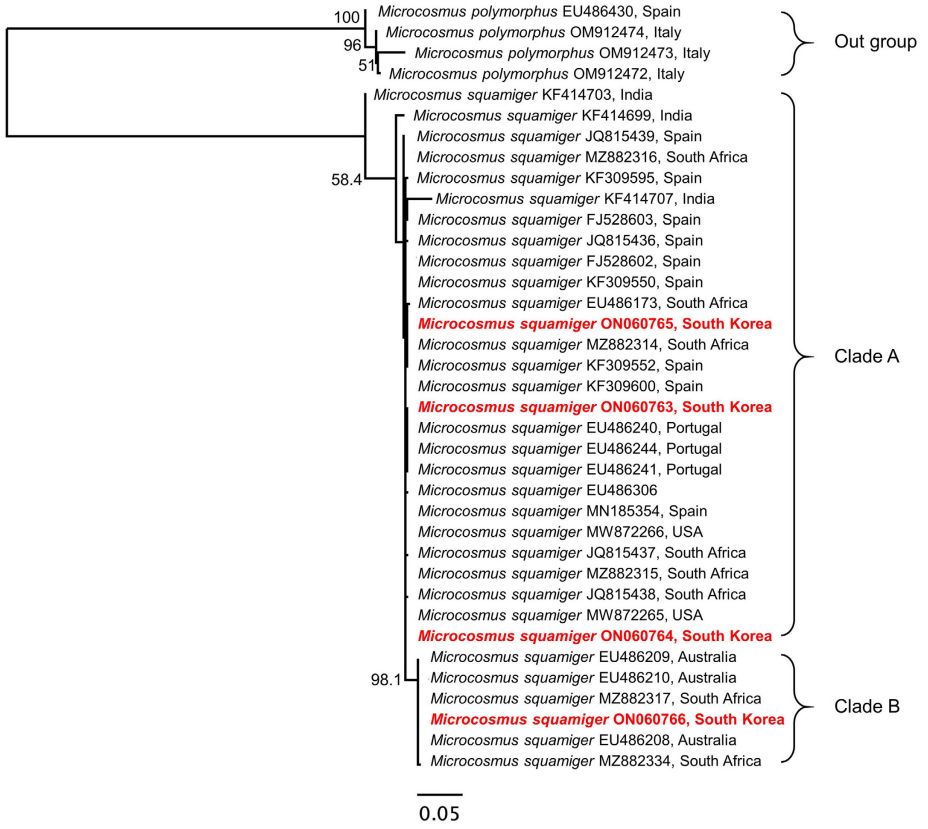

Figure 2. Maximum likelihood phylogenetic tree based on COI genetic sequences, showing the phylogenetic relationship between *M. squamiger* and the outgroup *M. polymorphus*. Bootstrap results from 1,000 replicates with > 50% support are shown on the branches. Sequences from this study are indicated in red and bold text.

and two from Wimi (ON060765 and ON060766) (Table 1). The BLAST results showed 98–100% sequence similarity with *M. squamiger* from other, worldwide specimens deposited into GenBank (Table S1) and its evolutionary relationship was depicted using a maximum likelihood (ML)-based phylogenetic tree. In concordance with previous studies (Rius et al. 2008), *M. polymorphus* was used as an outgroup, and the sequences obtained in our study were assorted as a group of sequences from *M. squamiger*. The *M. squamiger* sequences were divided into two clades (A and B). Out of the four nucleotide sequences used in this study, three were grouped in clade A and one was included in clade B (Figure 2).

Discussion

Morphological and phylogenetic identification

Microcosmus squamiger is an invasive ascidian species of Australian origin that has been detected in all continents except Antarctica (Rius et al. 2008; 2009). Our study is the first report of this species from Korea with morphological and phylogenetic verification of collected specimens for clear identification. As part of the morphological identification, the inner part of the siphons were compared with those in previous studies (Önen

2021), and the shape of dorsal tubercle with two spiral coils filling the peritubercular area was similar to that observed previously (Kott 1985; Monniot et al. 2001). Moreover, the siphon spines extended to approximately 15–20 μm in length with the overall shape of a roof tile (Monniot et al. 2001) or fingernail (Mastrototaro and Dappiano 2008; Önen 2021), which is consistent with previous findings. In addition, molecular identification was performed by using a maximum likelihood-based phylogenetic tree construction, which yielded two clades, similar to the results of a previous study (Rius et al. 2008). While clade A is more abundant in most parts of the world, including eastern Australia, clade B is relatively more abundant in western Australia (Rius et al. 2008). Since we studied only four nucleotide sequences, it was difficult to determine the relative abundance of the two clades in South Korea; therefore, further confirmation through future population studies is necessary.

Distribution and invasion

Microcosmus squamiger is currently distributed on most of the continents, but its existence in Asian countries has only been reported relatively recently. In India, it was first reported in 2006 (Abdul and Sivakumar 2007), although the authors did not provide clear taxonomic or genetic evidence. In Japan, which is adjacent to Korea, observations have been continuously reported since its first appearance at eight locations on the Pacific coast in 2007 (Nishikawa and Ueda 2011), and recently it was observed on Okinawa Island in 2014 (Nishikawa 2017). To our knowledge, this is the first report of *M. squamiger* from Korea.

No economic or biological damage has been reported from this species until recently. However, owing to its ability to form a dense community by attaching to hard substrates, fishing nets, ropes, etc. (Turon et al. 2007; Rius et al. 2009; Rius et al. 2012; Nishikawa 2017), *M. squamiger* is different from the ascidians previously reported from natural rocky areas in South Korea. In the present study, *M. squamiger* was found on an artificial structure (floating dock); therefore, it is unclear whether this species has expanded its habitat due to environmental changes or via anthropogenic activities. Therefore, additional research is required to discover the possible vector. Moreover, high density of *M. squamiger* is a potential hazard to fisheries, offshore structures, and indigenous species, and continuous monitoring studies are needed.

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Authors' contribution

Research conceptualization, C.-H.Y. and S.B.; Sample design and methodology, C.-H.Y. and S.B.; Investigation and data collection, C.-H.Y., S.B., S.-H.L. and J.M.K.; Data analysis and interpretation, C.-H.Y. and S.B.; Funding provision, C.-H.Y.; Roles/writing – original draft, S.B.; writing – review and editing, C.-H.Y. All authors have read and agreed to the published version of the manuscript.

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Supplementary material

The following supplementary material is available for this article:

Figure S1. *Microcosmus squamiger* morphological identification.

Table S1. Pairwise genetic distances between nucleotide sequences of COI sequences according to phylogenetic calculations.

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