



Short communication

Arrival of the non-indigenous brown alga *Mutimo cylindricus* to the Atlantic OceanD. Álvarez-Canali^a, M. Sansón^a, A. Tronholm^{a, b, c, *}^a BOTMAR-ULL, Departamento de Botánica, Ecología y Fisiología Vegetal, Universidad de La Laguna, 38200 La Laguna, Canary Islands, Spain^b Department of Biological and Environmental Sciences, University of Gothenburg, Box 463, Göteborg 405 30, Sweden^c Gothenburg Global Biodiversity Centre, Box 463, Göteborg 405 30, Sweden

ARTICLE INFO

Keywords:

Canary Islands
cox3
Cutleriaceae
Mutimo
Non-indigenous species
rbcL
Species distributions

ABSTRACT

Every introduction of a non-indigenous species (NIS) in coastal environments poses a threat to the native species and communities, as its effects in the ecosystem are not readily predictable. Introduction rates have kept increasing in the last decades, and our finding of the brown alga *Mutimo cylindricus* in the Canary Islands is another example of this general trend. This work represents the first record of the species outside its native range (Japan, Korea and the Philippines) in half a century, since its report in 1973 in Baja California, and marks the first report of *M. cylindricus* in the Atlantic Ocean. We analyzed the morphology of a male gametophyte observed in the Canary Islands and its phylogenetic relationship with other known populations using *rbcL* and *cox3* genes. The morphology of *M. cylindricus* in the Canary Islands is consistent with previous descriptions and the phylogenetic analyses revealed the close relationship with native populations from Japan. The finding of a male gametophyte is noteworthy, as introduced and some native populations of this species are composed exclusively by female gametophytes and thought to be parthenogenic, whereas androgenesis is considered rare in the field. Maritime traffic appears as the most apparent introduction vector of the species and it seems plausible that microscopic crustose sporophytes were present near the collecting site, highlighting the need of further monitoring of the invasive potential of this species in the Atlantic Ocean.

1. Introduction

The introduction of non-indigenous species (NIS) in marine habitats is a continuous process of species translocations that either intentionally or accidentally, directly or indirectly, involves human mediation (Streftaris et al., 2005). In the last decades, there has been an increase in the rate of introduction of marine NIS globally (Bailey et al., 2020), and every new introduced species poses a threat to native ecosystems as they can potentially become invasive, driving ecological changes that can lead to severe socioeconomic impacts (Pyšek et al., 2020). Early detection of NIS is crucial in order to manage their spread and impacts (Giakoumi et al., 2019); however, this task is usually hampered by the difficulties in species identification and taxonomic uncertainties in some groups (Hutchings, 2018). This applies especially to marine macroalgae, a group where molecular analyses are continuously revealing a high diversity of undescribed and cryptic species (McIvor et al., 2001; Zanolla et al., 2022) leading to changes in our understanding of its taxonomy and constantly updated geographic distributions (Golo et al., 2023).

An example of recent taxonomic changes is the family Cutleriaceae, a small group of brown algae (Phaeophyceae) that comprises only 14 species belonging to the genera *Cutleria* and the monotypic *Zanardinia* and *Mutimo*, which are mostly distributed in temperate and warm-temperate waters around the world (Guiry and Guiry, 2023). Most of these species are either known only from their original descriptions, or have restricted distributions and are recorded as introduced in part of their distribution range, such as *Cutleria multifida* (Turner) Greville and *Mutimo cylindricus* (Okamura) H. Kawai & T. Kitayama (Kogishi et al., 2010; Müller et al., 2011; Kawai et al., 2016). In the Atlantic Ocean, Cutleriaceae are represented by *Zanardinia typus* (Nardo) P.C. Silva and six *Cutleria* species: *C. adspersa* (Mertens ex Roth) De Notaris, *C. chilosa* (Falkenberg) P.C. Silva, *C. multifida*, *C. compressa* Kützinger, *C. laminaria* Kützinger and *C. pardalis* De Notaris, and the original descriptions are the only published data available for three latter (Guiry and Guiry, 2023). In particular, in the Canary Islands only three species are reported up until now, *C. chilosa*, *C. multifida* and *Z. typus*, all three considered native (Gobierno de Canarias, 2023) and exhibiting flat or compressed

* Corresponding author at: Department of Biological and Environmental Sciences, University of Gothenburg, Box 463, Göteborg 405 30, Sweden.
E-mail address: ana.tronholm@bioenv.gu.se (A. Tronholm).

morphology (Cormaci et al., 2012). However, recent surveys in the archipelago have allowed the detection of a species of Cutleriaceae with a distinct cylindrical morphology, which we herein aim to identify based on a combined morphological and molecular approach. This study represents the first record of *Mutimo cylindricus* outside its native range of Japan, Korea and the Philippines (Okamura, 1902; Cotton, 1906; Silva et al., 1987) in half a century, since its report in 1973 in Baja California (Hollenberg, 1978), and marks the first report of *M. cylindricus* in the Atlantic Ocean.

2. Material and methods

A single individual of *Mutimo cylindricus* was collected by hand in Tenerife (Canary Islands) (Teresitas Beach; 28.509099°N, 16.181009°W) on 26 March 2022, growing on a surface buoy, and immediately transported in a cooler with seawater to the laboratory (ca. 20 km away). A subsample was preserved in silica gel for molecular analysis and the remaining material was fixed in a 4 % formalin-seawater solution for morphological analysis. A voucher was subsequently deposited in the herbarium of the University of La Laguna, Canary Islands, Spain, TFC Phyc 16432. Morphological identification was based on habit and external morphology, observed under a Leica EZ4 stereomicroscope (Leica Microsystems, Germany), and on the anatomy, using a Leica DM750 microscope (Leica Microsystems, Germany). Total genomic DNA was extracted using DNeasy PowerPlant Pro Kit (Qiagen, Germany) according to manufacturer's instructions, and subsequently purified with DNeasy PowerClean Pro Cleanup Kit (Qiagen, Germany). Mitochondrial cytochrome oxidase subunit III (*cox3*) and chloroplastic ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (*rbcl*) were amplified following Mattio et al. (2008) and Bittner et al. (2008). PCR products were purified using QIAquick PCR Purification Kit (Qiagen, Germany) following manufacturer's recommendations, and sequencing was performed at Eurofins Genomics Europe.

A concatenated alignment of 17 species (16 ingroup and 1 outgroup) and 32 sequences (15 *cox3* and 17 *rbcl*) was constructed including the newly generated sequences in addition to all publicly available sequences of Cutleriaceae in GenBank for those markers (see Table S1 in supplementary material). The sequences were aligned using Clustal Omega and manually curated. For each gene, the best-fitting nucleotide substitution model was selected using jModelTest 2.1.7 (Darriba et al., 2012) with Akaike Information Criteria. Maximum Likelihood (ML) analysis was performed using RAxML 8.2.11 in Geneious Prime 2023.1.2 (Dotmatrix, UK). A rapid bootstrap analysis and search for the best-scoring ML tree was performed for one single run with 1000 bootstrap replicates under GTR+G+I model partitioned for each gene. Bayesian Inference analysis was performed using MrBayes 3.2.7 implemented at the CIPRES Science Gateway 3.3. (Miller et al., 2010). Two independent runs of four chains of Markov chain Monte Carlo iterations were run simultaneously for 10^6 generations, sampling every 100 generations and a 10 % burn-in, under GTR+G+I model partitioned for each gene.

3. Results and discussion

The individual of *Mutimo cylindricus* observed in the Canary Islands was up to 12 cm in total length and yellow brown in color. The specimen was attached to the substratum by a small conical holdfast from which two erect main axes were formed. Main axes were cylindrical, up to 1.6 mm in diameter, tapering towards the basal and apical parts, regularly dichotomously branched (Fig. 1a), with slender apices up to 140 μ m in diameter and bearing deciduous assimilatory hairs in the apical portions. The typical trichothallic growth was observed, with turfs of hairs up to 1.8 mm long and 17 μ m in diameter in the apices (Fig. 1b) that were initially uniseriate and became multiseriate by longitudinal divisions of the cells (Fig. 1c). In cross-section, the main axes showed the parenchymatous structure, with a medulla of round cells up to 36 μ m in

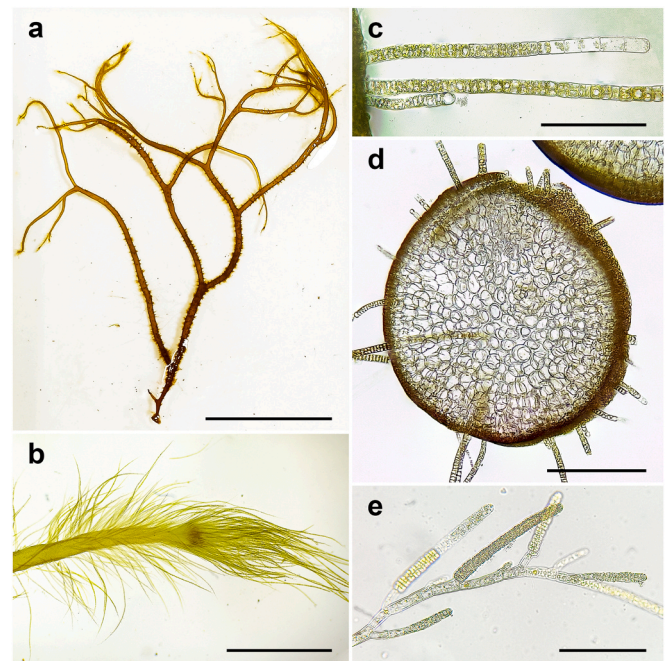


Fig. 1. Morphology of male gametophyte of *Mutimo cylindricus* collected in the Canary Islands. a) Habit. b) Detail of an apical portion, showing the apical hair turf and deciduous assimilatory hairs. c) Detail of assimilatory hairs with uniseriate and multiseriate portions. d) Cross-section of an apical portion of the thallus showing the parenchymatous structure. e) Detail of a fertile filament bearing spermatangia in different maturity stages. Scale bar: a = 3 cm; b = 1 mm; c, e = 100 μ m; d = 200 μ m.

diameter that could become hollow in the center, and were surrounded by a layer of smaller, heavily pigmented cortical cells (Fig. 1d). All these morphological characters were congruent with the description of *M. cylindricus*, except for a smaller thallus size compared to descriptions from other regions (Okamura, 1902; Hollenberg, 1978).

The collected individual was a male gametophyte, with abundant sori scattered up to 2/3 of its total length, formed by dense turf of fertile filaments, each bearing several pale, cylindrical plurilocular sporangia (Fig. 1e). The finding of a male gametophyte is noteworthy, since the introduced populations in Baja California are composed exclusively by female gametophytes, and are thought to be parthenogenic, as some populations in Japan (Kogishi et al., 2010). Androgenesis is known to take place in laboratory conditions, but it is considered rare in the field (Kitayama et al., 1992). The development of gametophytes in *M. cylindricus* is highly seasonal, being reported only during the cold months, from late winter to early spring (Lee et al., 2001; Kim et al., 2008; Miller et al., 2011). This pattern was also observed in this study as the specimen was found in March, following the lowest annual sea surface temperature values in the Canary Islands (Barton et al., 1998).

Phylogenetic analyses confirmed the identity of *Mutimo cylindricus*, as the sequences from the Canary Islands clustered together with the sequences from Japan and South Korea in a fully supported clade (Fig. 2). The new sequences showed 99.9 % (*rbcl*) and 100 % (*cox3*) identity with available *M. cylindricus* sequences. The *cox3* gene has previously been used for population genetics in this species, and our sequence is identical to haplotypes detected only in Japan according to Kogishi et al. (2010), pointing to a direct introduction from its native range. Finding an Asiatic species in the Atlantic Ocean is not as uncommon as it might appear, as similar events have happened in the past. For example, *Papenfussiella kuromo* (Yendo) Inagaki, *Grateloupia imbricata* Holmes and *Pachymeniopsis gargiuloi* S.Y. Kim, Manghisi, Morabito & S.M. Boo, whose native distribution spans Japan and China, were observed in the Canary Islands between the early 90s and late 2000

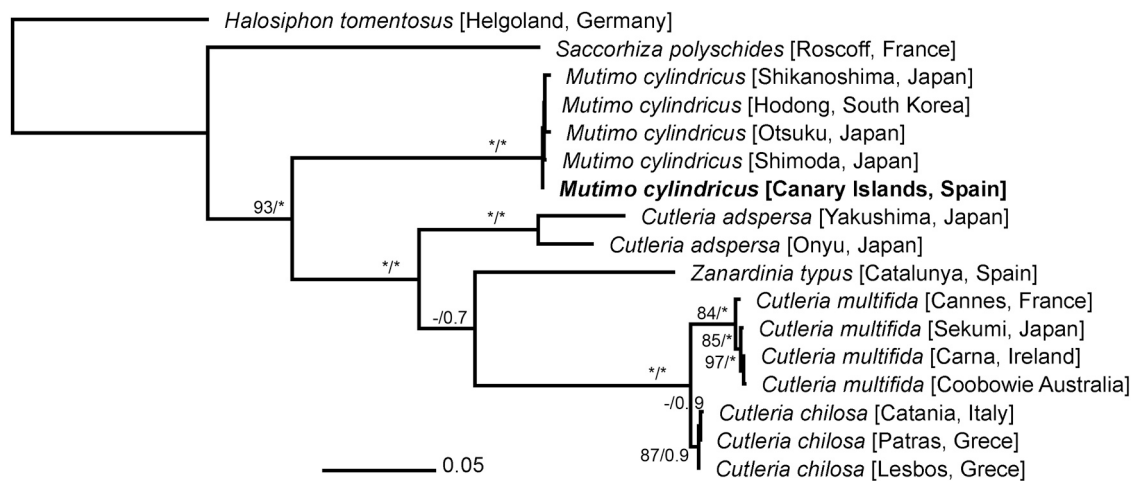


Fig. 2. Phylogenetic tree of a concatenated *cox3* and *rbcL* partial genes of Cutleriaceae. Numbers at nodes indicate bootstrap values of Maximum Likelihood (ML) analysis (right) and posterior probabilities of Bayesian Inference (BI) analysis (left).

(Martín et al., 1996; García-Jiménez et al., 2008). The detection rate of introduced species has been increasing in the Canary Islands since the 90s, and it is reported that about 10% of the non-indigenous marine species observed in this region come from the NW Pacific (Castro et al., 2022). Many of those have been discovered in or near marinas in this archipelago in the past (Afonso-Carrillo et al., 2003), which has partially been explained by the high international maritime traffic of the main ports of the archipelago (Castro et al., 2022). Our study site (Teresitas Beach) is <5 km away from the main harbor in Tenerife (i.e. Santa Cruz), where other macroalgae, such as *Predeaa huismannii* Kraft, have first been detected (Sansón et al., 1991). Thus maritime traffic appears as the most apparent introduction vector of *M. cylindricus* in the Canary Islands.

Mutimo cylindricus is not considered an invasive species in California, where it is well established since 1973, as it has kept a limited distribution and has not caused ecological disturbances over 50 years (Miller et al., 2011). Species traits and previous invasive history have been used to predict its behavior in a new introduction event (Nyberg and Walentinus, 2005; Sarà et al., 2013). However, the extrapolation of these results requires caution, as the conditions are not equal in every environment nor in every introduction event, and could lead to disparate consequences (Geburzi and McCarthy, 2018). Even though only a single specimen was detected in the Canary Islands, it seems plausible that microscopic crustose sporophytes were present near the collecting site as they are easily overlooked, highlighting the need of further monitoring of the invasive potential of this species in the Atlantic Ocean.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data will be made available on request.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.aquabot.2023.103745.

References

- Afonso-Carrillo, J., Sansón, M., Reyes, J., González-Rojas, B., 2003. Morfología y distribución de la rodófito alóctona *Neosiphonia harveyi*, y comentarios sobre otras algas marinas probablemente introducidas en las Islas Canarias. *Rev. de la Acad. Canar. de Cienc.* 14 (3–4), 83–98.
- Bailey, S.A., Brown, L., Campbell, M.L., Canning-Clode, J., Carlton, J.T., Castro, N., Chainho, P., Chan, F.T., Creed, J.C., Curd, A., Darling, J., Fofonoff, P., Galil, B.S., Hewitt, C.L., Inglis, G.J., Keith, I., Mandrak, N.E., Marchini, A., McKenzie, C.H., Zhan, A., 2020. Trends in the detection of aquatic non-indigenous species across global marine, estuarine and freshwater ecosystems: a 50-year perspective. *Divers. Distrib.* 26 (12), 1780–1797. <https://doi.org/10.1111/ddi.13167>.
- Barton, E.D., Aristegui, J., Tett, P., Cantón, M., García-Braun, J., Hernández-León, S., Nykjaer, L., Almeida, C., Almunia, J., Ballesteros, S., Basterretxea, G., Escánez, J., García-Weill, L., Hernández-Guerra, A., López-Laatzén, F., Molina, R., Montero, M. F., Navarro-Pérez, E., Rodríguez, J.M., Wild, K., 1998. The transition zone of the canary current upwelling region. *Prog. Oceanogr.* 41 (4), 455–504. [https://doi.org/10.1016/S0079-6611\(98\)00023-8](https://doi.org/10.1016/S0079-6611(98)00023-8).
- Bitner, L., Payri, C.E., Couloux, A., Cruaud, C., De Reviere, B., Rousseau, F., 2008. Molecular phylogeny of the Dictyotales and their position within the Phaeophyceae, based on nuclear, plastid and mitochondrial DNA sequence data. *Mol. Phylogenet. Evol.* 49 (1), 211–226. <https://doi.org/10.1016/j.ympev.2008.06.018>.
- Castro, N., Carlton, J.T., Costa, A.C., Marques, C.S., Hewitt, C.L., Cacabelos, E., Lopes, E., Gizzi, F., Gestoso, I., Monteiro, J.G., Costa, J.L., Parente, M., Ramalhosa, P., Fofonoff, P., Chainho, P., Haroun, R., Santos, R.S., Herrera, R., Marques, T.A., Canning-Clode, J., 2022. Diversity and patterns of marine non-native species in the archipelagos of Macaronesia. *Divers. Distrib.* 28 (4), 667–684. <https://doi.org/10.1111/ddi.13465>.
- Cormaci, M., Furnari, G., Catra, M., Alongi, G., Giaccone, G., 2012. Flora marina bentonica del Mediterraneo: Phaeophyceae. *Boll. Dell'accademia Gioenia Di Sci. Nat. Di Catania* 45 (375), 1–508.
- Cotton, A.D., 1906. Marine algae from Corea. *Bull. Misc. Inf. (R. Bot. Gard., Kew)* 1906 (9), 366–373.
- Darriba, D., Taboada, G.L., Doallo, R., Posada, D., 2012. jModelTest 2: more models, new heuristics and parallel computing. *Nat. Methods* 9 (8), 772–773. <https://doi.org/10.1038/nmeth.2109>.
- García-Jiménez, P., Geraldino, P.J.L., Boo, S.M., Robaina, R.R., 2008. Red alga *Grateloupia imbricata* (Halymeniaceae), a species introduced into the Canary Islands. *Phycol. Res.* 56 (3), 166–171. <https://doi.org/10.1111/j.1440-1835.2008.00498.x>.
- Geburzi, J.C., McCarthy, M.L., 2018. How do they do it? – Understanding the success of marine invasive species. In: Jungblut, S., Liebich, V., Bode, M. (Eds.), *YOUARES 8 – Oceans Across Boundaries: Learning from Each Other*. Springer International Publishing, pp. 109–124. https://doi.org/10.1007/978-3-319-93284-2_8.
- Giakoumi, S., Katsanevakis, S., Albano, P.G., Azzurro, E., Cardoso, A.C., Cebrian, E., Deidun, A., Edelist, D., Francour, P., Jimenez, C., 2019. Management priorities for marine invasive species. *Sci. Total Environ.* 688, 976–982. <https://doi.org/10.1016/j.scitotenv.2019.06.282>.
- Gobierno de Canarias. (2023). Banco de Datos de Biodiversidad de Canarias. <https://www.biodiversidadcanarias.es/biota/> [accessed 10 July 2023].
- Golo, R., Vergés, A., Díaz-Tapia, P., Cebrian, E., 2023. Implications of taxonomic misidentification for future invasion predictions: Evidence from one of the most harmful invasive marine algae. *Mar. Pollut. Bull.* 191, 114970. <https://doi.org/10.1016/j.marpollbul.2023.114970>.
- Guiry, M.D., & Guiry, G.M. (2023). Algaebase. World-wide electronic publication, National University of Ireland, Galway. <https://www.algaebase.org> [accessed 10 July 2023].
- Hollenberg, G.J., 1978. Phycological notes VIII. Two brown algae (Phaeophyta) new to California. *Bulletin. South. Calif. Acad. Sci.* 77 (1), 28–35.

- Hutchings, P., 2018. Marine introduced species in Australia, where to from here? A personal perspective from a practising taxonomist. *Mar. Pollut. Bull.* 136, 477–480. <https://doi.org/10.1016/j.marpolbul.2018.09.047>.
- Kawai, H., Kogishi, K., Hanyuda, T., Arai, S., Gurgel, C.F., Nelson, W., Meinesz, A., Tsiamis, K., Peters, A.F., 2016. Phylogeographic analysis of the brown alga *Cutleria multifida* (Tilopteridales, Phaeophyceae) suggests a complicated introduction history. *Phycol. Res.* 64 (1), 3–10. <https://doi.org/10.1111/pre.12113>.
- Kim, M.-S., Kim, M.-R., Chung, M.-H., Kim, J.-H., Chung, I.-K., 2008. Species composition and biomass of intertidal seaweeds in Chuja Island. *Algae* 23 (4), 301–310. <https://doi.org/10.4490/algae.2008.23.4.301>.
- Kitayama, T., Kawai, H., Yoshida, T., 1992. Dominance of female gametophytes in field populations of *Cutleria cylindrica* (Cutleriales, Phaeophyceae) in the Tsugaru Strait, Japan. *Phycologia* 31 (5), 449–461. <https://doi.org/10.2216/i0031-8884-31-5-449.1>.
- Kogishi, K., Kitayama, T., Miller, K.A., Hanyuda, T., Kawai, H., 2010. Phylogeography of *Cutleria cylindrica* (Cutleriales, Phaeophyceae) in northeastern Asia, and the identity of an introduced population in California. *J. Phycol.* 46 (3), 553–558. <https://doi.org/10.1111/j.1529-8817.2010.00818.x>.
- Lee, J.W., Kim, Y.H., Lee, H.-B., 2001. The community structure of intertidal marine benthic algae in the east coast of Korea. *Algae* 16 (1), 113–118.
- Martín, M.J., Sansón, M., Reyes, J., 1996. Morphology and anatomy of *Papenfussiella kuromo* (Chordariaceae, Phaeophyta) from the Canary Islands. *Cryptogam. Algol.* 17 (3), 165–173.
- Mattio, L., Payri, C.E., Stiger-Pouvreau, V., 2008. Taxonomic revision of *Sargassum* (fucales, Phaeophyceae) from French polynesia based on morphological and molecular analyses1. *J. Phycol.* 44 (6), 1541–1555. <https://doi.org/10.1111/j.1529-8817.2008.00597.x>.
- McIvor, L., Maggs, C.A., Provan, J., Stanhope, M.J., 2001. *rbcL* sequences reveal multiple cryptic introductions of the Japanese red alga *Polysiphonia harveyi*. *Mol. Ecol.* 10 (4), 911–919. <https://doi.org/10.1046/j.1365-294X.2001.01240.x>.
- Miller, K.A., Aguilar-Rosas, L.E., Pedroche, F.F., 2011. A review of non-native seaweeds from California, USA and Baja California, Mexico. *Hidrobiologica* 21 (3), 365–379.
- Miller, M.A., Pfeiffer, W., Schwartz, T., 2010. Creating the CIPRES science gateway for inference of large phylogenetic trees. *Gatew. Comput. Environ. Workshop (GCE)* 2010, 1–8. <https://doi.org/10.1109/GCE.2010.5676129>.
- Nyberg, C.D., Wallentinus, I., 2005. Can species traits be used to predict marine macroalgal introductions? *Biol. Invasions* 7 (2), 265–279. <https://doi.org/10.1007/s10530-004-0738-z>.
- Okamura, K. (1902). *Nihon kaiso zuzetsu. Illustrations of the marine algae of Japan* (Vol. 1). Keigyosha & Co.
- Pyšek, P., Hulme, P.E., Simberloff, D., Bacher, S., Blackburn, T.M., Carlton, J.T., Dawson, W., Essl, F., Foxcroft, L.C., Genovesi, P., Jeschke, J.M., Kühn, I., Liebhold, A.M., Mandrak, N.E., Meyerson, L.A., Pauchard, A., Pergl, J., Roy, H.E., Seebens, H., Richardson, D.M., 2020. Scientists' warning on invasive alien species. *Biol. Rev.* 95 (6), 1511–1534. <https://doi.org/10.1111/brv.12627>.
- Sansón, M., Reyes, J., Afonso-Carrillo, J., 1991. Contribution to the seaweed flora of the Canary Islands: new records of Florideophyceae. *Bot. Mar.* 34 (6), 527–536. <https://doi.org/10.1515/botm.1991.34.6.527>.
- Sarà, G., Palmeri, V., Rinaldi, A., Montalto, V., Helmuth, B., 2013. Predicting biological invasions in marine habitats through eco-physiological mechanistic models: a case study with the bivalve *Brachidontes pharaonis*. *Divers. Distrib.* 19 (10), 1235–1247. <https://doi.org/10.1111/ddi.12074>.
- Silva, P.C., Meñez, E.G., Moe, R.L., 1987. *Catalog of the Benthic Marine Algae of the Philippines*. Smithsonian Institution Press.
- Streftaris, N., Zenetos, A., & Papatthanassiou, E. (2005). Globalisation in marine ecosystems: The story of non-indigenous marine species across European seas. In *Oceanography and marine biology* (pp. 429–464). CRC Press.
- Zanolla, M., Carmona, R., Mata, L., De la Rosa, J., Sherwood, A., Barranco, C.N., Muñoz, A.R., Altamirano, M., 2022. Concise review of the genus *Asparagopsis* Montagne, 1840. *J. Appl. Phycol.* 34 (1), 1–17. <https://doi.org/10.1007/s10811-021-02665-z>.