

NOTE

GRACILARIA VERMICULOPHYLLA (RHODOPHYTA, GRACILARIALES) IN HOG ISLAND BAY, VIRGINIA: A CRYPTIC ALIEN AND INVASIVE MACROALGA AND TAXONOMIC CORRECTION¹

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***Gracilaria* in Virginia, USA, is abundant and composed of thalli either having relatively flat or cylindrical branches. These two morphologies were referred to previously as *G. foliifera* (Forsskål) Børgesen and *G. verrucosa* (Hudson) Papenfuss. However, *G. verrucosa* is regarded an invalid name, and the flat specimens are now referred to as *G. tikvahiae* McLachlan. This has created confusion about the nomenclature of *Gracilaria* from this region. Here we document that the cylindrical form that dominates Hog Island Bay, Virginia, is *G. vermiculophylla* (Ohmi) Papenfuss, an alien macroalga from the West Pacific. Most of the ecological studies performed at the Long Term Ecological Research (LTER) site in Hog Island Bay used this cylindrical species. The present study clarifies the taxonomical status of this species, and we identify attributes that make this alien successful in turbid coastal lagoons.**

Key word index: alien macroalgae; cryptic invasion; *Gracilaria vermiculophylla*; phylogeography; Virginia

Abbreviations: cox, cytochrome oxidase; ITS, internal transcribed spacer; *rbcL*, large subunit of the RUBISCO gene

The genus *Gracilaria* (Gracilariales, Rhodophyta) has a worldwide distribution, comprising more than 110 species (Gurgel and Fredericq 2004), of which many are important economically (Oliveira and Plastino 1994, Oliveira et al. 2000) and ecologically (Mc

Lachlan and Bird 1986). Unfortunately, in many cases, *Gracilaria* species are difficult to identify based on morphological features (Oliveira et al. 2000, Gurgel and Fredericq 2004). Given these difficulties and new possibilities of accurate identification by molecular biology techniques, *Gracilaria sensu lato* species are regularly changing taxonomic status (Bird and Rice 1990, Bellorin et al. 2002, Gurgel and Fredericq 2004, Gurgel et al. 2004).

Gracilaria is a particularly important genus in Virginia, USA, where it is abundant in lagoons and estuaries (Humm 1979, Thomsen 2004a). In Virginia, cylindrical *Gracilaria* specimens have been referred to traditionally as *G. verrucosa* (Hudson) Papenfuss, whereas flat specimens have been referred to as *G. foliifera* (Forsskål) Børgesen (1932) or *G. foliifera* var. *angustissima* Taylor (1937) (Zaneveld and Barnes 1965, Mangum et al. 1968, Rhodes 1970, Humm 1979, Connor 1980, Parker et al. 2001), based on the work of Taylor (1957, 1960) and Humm (1979), who cited these species for the western Atlantic. This taxonomy has been used in several recent studies from Hog Island Bay, Virginia (Thomsen 2004a, b, Thomsen and McGlathery 2005). However, Steentoft et al. (1991) and Irvine and Steentoft (1995) have shown that the *G. verrucosa* epithet is taxonomically invalid. In addition, based on DNA analysis of nuclear ITS *rDNA* and the chloroplast *rbcL* gene, the species identified as *G. foliifera* is known today as *G. tikvahiae* McLachlan (Bird and Rice 1990, Gurgel and Fredericq 2004, Gurgel et al. 2004). This has led several Hog Island Bay studies to refer to all *Gracilaria* material as *G. tikvahiae* (McGlathery et al. 2001, Tyler et al. 2001, Tyler 2002, Anderson et al. 2003, Tyler and McGlathery 2003). It is noteworthy that most material in these studies was composed of cylindrical *Gracilaria* specimens. Still, the cylindrical forms from Virginia remain a taxonomic

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problem, and when only character-poor specimens are available, molecular analysis remains the most reliable species identifier.

In 1999, a single cylindrical *Gracilaria* specimen from Hog Island Bay was *rbcL*-analyzed (GenBank AY049312, Gurgel and Fredericq 2004), and subsequent phylogenetic analyses by Rueness (2005) indicated that this specimen could be *G. vermiculophylla* (Ohmi) Papenfuss, a species of Northwestern Pacific origin, which is an invader in the east Pacific (Bellorin et al. 2004) and in several European countries (Rueness 2005). The goal of the present study was to confirm the status of the cylindrical Hog Island Bay *Gracilaria* species. Cylindrical *Gracilaria* samples were collected on 8 December 2004 from three key localities used to study the ecology of Hog Island Bay: Little Cobb Island (37°18.262'N, 75°47.569'W), Oyster Harbor (37°17.306'N, 75°55.399'W), and South end of Hog Island (37°23.218'N, 75°43.322'W). Algal samples were freeze dried and stored in plastic bags with silica gel. Vouchers were fixed in 4% formalin/seawater solution and herbarium pressed. Exsiccate were deposited in the herbarium of the University of Louisiana at Lafayette. The DNA was extracted with a Plant Dneasy MiniKit (Qiagen, Valencia, CA, USA) following the manufacturer's protocol. The *cox2-cox3* mitochondrial DNA spacer region was amplified following protocol and primers as described by Zuccarello et al. (1999). The PCR amplifications were cleaned with ExoSAP-IT® (USB Corp., Cleveland, OH, USA) following the manufacturer's protocol. Both strands of the *cox2-cox3* spacer were sequenced using the same PCR primers and BigDye Terminator chemistry (Applied Biosystems, Foster City, CA, USA) following the manufacturer's protocol. The DNA sequences were assembled with a Sequencher (Gene Codes Corp., Ann Arbor, MI, USA) and aligned manually in a nexus file together with the published *cox2-cox3* DNA sequences from GenBank.

All three samples provided identical *cox2-cox3* spacer mitochondrial DNA sequences (Genbank DQ173302, DQ173303, DQ173304). This single haplotype was also 100% identical to the single haplotype sequenced for 22 specimens studied by Rueness (2005) in northern Europe (France AY725141 and AY725152-63), Sweden (AY725147), Spain (AY725143), Portugal (AY725146), and the Netherlands (AY725142), including one sample from South Korea (AY725144), and one from Japan (AY725145). This confirmed that the cylindrical *Gracilaria* that dominates Hog Island Bay is indeed *G. vermiculophylla*, and that all samples from the northern Atlantic analyzed so far present no genetic differences with regard to *cox2-cox3* spacer DNA sequences. Our samples also matched morphological descriptions of *Gracilaria vermiculophylla* as described in Terada and Yamamoto (2002) and Rueness (2005). Anatomical features of vegetative (i.e. spherical medullary cells, abrupt transition between the medulla and the cortex, and anticlinally elongated cortical cells) and cystocarpic characters (i.e. rarity of traversing fila-

ments, overall gonimoblast mass with a triangular shape and lobed contour, carposporangia organized in distinct chains with progressively maturing stages toward the apice) corresponded exactly to the ones observed in these two works. *Gracilaria vermiculophylla* probably arrived in Virginia attached to transplanted oysters, because it is an efficient recruiter onto bivalve shells and man-made structures, and has a high ability to recover from fragmentation (Thomsen 2004a, b). It is unlikely to have drifted over long distances because of its negative buoyancy. In Hog Island Bay, *G. vermiculophylla* has been the dominant macroalga at least since 1998 (Thomsen 2004a) when our intensive LTER studies began. Now that we have established that the taxonomical status of the cylindrical *Gracilaria* in Hog Island Bay is *G. vermiculophylla*, our previous work provides insights into its success as an invader. Hog Island Bay is a turbid shallow lagoon, where *G. vermiculophylla* represents >80% of the submerged macrophyte biomass. We suggest that its dominance in the lagoon is related to the following: (1) its association with the ubiquitous polychaete, *Diopatra cuprea*, (2) its efficient recruitment on hard substratum, (3) its high tolerance of desiccation, grazing, burial by sediment, low light, and low and high temperatures, and (4) its long-term persistence as both small and large vegetative fragments (as *G. verrucosa*, Thomsen 2004a, b, Thomsen and McGlathery 2005, in press).

To avoid future taxonomic confusion, we encourage researchers to create silica-gel, air-dried, and/or herbarium presses as voucher specimens so that the correct identification can be confirmed using morphological and molecular analysis. Such precautions need to be undertaken to avoid similar cryptic invasions, such as that which also occurred with *Codium fragile* ssp. *tomentosoides* (Trowbridge 1998).

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