Cryptogamie, Algologie, 2016, 37 (4): 257-263

New record of the non-native seaweed *Gracilaria parvispora* in Baja California – A note on Vergara-Rodarte *et al.* (2016)

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Abstract – The delimitation of species in the Gracilariales is often difficult due to the lack of diagnostic morphological characters. As a result, non-native species are often misidentified without the use of molecular tools. Recently, studies have investigated the agar quality of the dominant gracilarioid species in the Laguna San Ignacio in Baja Califronia Sur, Mexico, including Vergara-Rodarte *et al.*, (2016) published in the May edition of *Cryptogamie, Algologie*. The species has been reported as *Gracilaria pacifica, G. vermiculophylla* and *Gracilariopsis* sp. Using a combination of three mitochondrial and plastid markers, we identified this species as *G. parvispora*, extending the known distribution of this non-native species in Baja California. Due to the potential of *G. parvispora* as a source of agar, more detailed studies of on the invasion history and surveys are necessary in the eastern Pacific to determine its current distribution and impacts on the native biodiversity.

Baja California / Biological invasión / *cox*1 / *cox*2-3 spacer / *Gracilaria* / *rbc*L / Species delimitation

The delimitation of gracilarioid algae is notoriously fraught with problems due to the lack of diagnostic morphological characters (Bird, 1995) and, as a result, can often lead to species misidentification (e.g., Cohen *et al.,* 2004). Non-native species in the family Gracilariaceae often go unnoticed until molecular tools are used to determine taxonomic identity. For example, in coastal bays and estuaries in Virginia, cylindrical specimens were identified as either *Gracilaria verrucosa* (Hudson) Papenfuss (now considered a taxonomic synonym of *Gracilariopsis*

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doi/10.7872/crya/v37.iss4.2016.257

longissima (S.G. Gmelin) M. Steentoft, L.M. Irvine & W.F. Farnham) or *G. tikvahiae* McLachlan (native to the eastern coast of the United States). However, ecological studies at the Long Term Ecological Monitoring site in Hog Island Bay were in fact assessing the impacts of the invasive *G. vermiculophylla* (Ohmi) Papenfuss (Thomsen *et al.,* 2006), a species widely introduced in the Northern Hemisphere from the northwest Pacific (Kim *et al.,* 2010a; Krueger-Hadfield *et al.,* 2016a, 2016b).

Accurate species identification is critical in order to understand the impacts of non-native species on native biodiversity. Species of economic interest, such as *Undaria pinnatifida* (Harvey) Suringar (Grulois *et al.*, 2011), *Kappaphycus alvarezii* (M.S. Doty) M.S. Doty *ex* P.C. Silva (Sellers *et al.*, 2015) or *Gracilaria salicornia* (C. Agardh) Dawson (Smith *et al.*, 2004), have been intentionally introduced around the world for aquaculture industries and have subsequently "escaped the farm". Similarly, there are algae used in aquaria by professionals and hobbyists, such as the genus *Caulerpa*, that have since found their way into nearby ecosystems (Stam *et al.*, 2006).

Recent studies have reported the presence of two invasive gracilarioid species, *Gracilaria vermiculophylla* and *G. parvispora* (C. Agardh) Dawson in Baja California, Mexico (Bellorin *et al.*, 2004; Miller *et al.*, 2012; García-Rodríguez *et al.*, 2013). Kim *et al.*, (2010a) and Krueger-Hadfield *et al.*, (2016b) have documented the spread of *G. vermiculophylla* throughout the Northern Hemisphere. However, the extent of the native range of *G. parvispora* and its status as native or non-native has been debated. Abbott (1999) had suggested that *G. parvispora* was an endemic to Hawai'i. Nelson *et al.*, (2009), by contrast, hypothesized that that *G. parvispora* Abbott (type locality: Kaneohe Bay, Oahu, Hawai'i) is a native to Asia, perhaps Japan, and was possibly either introduced to Hawaii or was a narrow endemic until it was intentionally introduced around the islands for aquaculture. Nelson *et al.*, (2009) suggested a Japanese origin of *G. parvispora* in Hawai'i by citing evidence including the earliest Bishop Museum record is from 1908 (though this has not been confirmed to our knowledge with molecular methods), the lack of a Hawaiian name, the availability in Hawaiian fish markets since around 1945 and its disjunct distribution around the Hawaiian Islands. However, unlike *Kappaphycus* or *G. salicornia* (see Smith *et al.*, 2004 for a discussion of these species), there is no actual evidence of human introduction of this taxon to Hawai'ian waters. Moreover, Kim *et al.*, (2008) asserted that *Gracilaria bursa-pastoris* (S. Gmelin) Silva (type locality: Mediterranean Sea) reported from Korea and Japan was a misidentification of *G. parvispora*, basing their conclusion on *rbc*L sequences from Hawai'i, South Korea, Japan and Italy in which the sequences from South Korea, Japan and Hawai'i fell in a different clade from that of the Italian specimen. As a result, the distribution of *G. parvispora* should include central and western Pacific waters.

In northwestern Mexico, despite being large and conspicuous, *Gracilaria vermiculophylla* and *G. parvispora* were either misidentified as congeneric native species (e.g., Bellorin *et al.*, 2004) or were recorded at single sites without molecular confirmation (e.g., Dreckman, 1999). *Gracilaria vermiculophylla* has been present in Baja California since at least 1979 (Bellorin *et al.*, 2004), whereas, *G. parvispora* was not reported until 1999 (Dreckman, 1999). However, first records in taxonomically difficult genera may reflect the first time a specimen was collected, rather than the date at which the introduction occurred. Several introduced gracilarioid seaweeds in North America have been present, but unrecognized, decades longer than published first records (S.A. Krueger-Hadfield and K.A. Miller, *unpublished data*). For both *G. vermiculophylla* and *G. parvispora*, detailed study of archival herbarium collections and contemporary surveys are necessary in order to determine the distribution of these species outside the northwest Pacific, particularly with regard to estimating the timing of the invasions.

Both *Gracilaria vermiculophylla* and *G. parvispora* have or have had some economic importance. The former was an important species in the agar industry in Japan (Okazaki, 1971) and the latter is an important alga in Hawaiian cuisine (Glenn *et al.*, 1996, Abbott, 1999). Though it is uncertain whether aquarium shops sell *G. parvispora*, or some mixture of different gracilarioid seaweeds, many E-commerce sites purport to sell this species (see for example, http://marineplantbook.com). Because both species are often found together in northwestern Mexico, it is possible that they have been intentionally introduced for use in marine aquaria or for cultivation (García-Rodríguez *et al.*, 2013).

A gracilarioid seaweed, reported both as *G. vermiculophylla* and *Gracilariopsis* species, has used in studies of agar quality and biomass in the Laguna San Ignacio in Baja California Sur, Mexico (Vergara-Rodarte *et al*., 2010, 2016). This species demonstrated high yield (17%) and gel strength (1,132 g cm^{-2}) after shorter alkaline treatments, indicating that it is suitable for agar production (Vergara-Rodarte *et al.*, 2010). Vergara-Rodarte *et al.*, (2016) assessed the population structure and standing crop (1,004 wet tons) of what they identified as a *Gracilariopsis* species in the same lagoon. However, the morphology of the spermatangial conceptacle (see Figure 2 from Vergara-Rodarte *et al*., 2016) indicated that the species in Laguna San Ignacio belongs to the genus *Gracilaria* rather than *Gracilariopsis* (Kim *et al*., 2008, R. Terada, *pers. obs.*).

In order to resolve the identity of this potentially valuable gracilariod seaweed, we used the mitochondrial barcode markers *cox*1 and *cox*2-3 spacer and the plastid *rbc*L marker to genotype a representative specimen sampled from El Cordón in Laguna San Ignacio (26º48'46''N 113º12'23''W) in January 2016. We extracted DNA using the DNeasy Blood and Tissue kit (Qiagen, Valencia, CA, USA) following the protocol adapted by Lindstrom *et al.*, (2011). The *cox*1 and *cox*2-3 spacer were amplified using the primer pairs 622F (Yang *et al.*, 2008) and 1549R (Geraldino *et al.*, 2006) and cox2-for and cox3-rev (Zuccarello *et al.*, 1999), respectively. The *rbc*L was amplified using the primer pair F57_rbcL and rbcLrevNEW (Saunders & Moore, 2013). PCR programs were described in Yang *et al.*, (2008) for *cox*1, in Zuccarello *et al*., (1999) for *cox*2-3 and Saunders and Moore, (2013) and the amplification followed protocol described in Krueger-Hadfield *et al.*, (2016b) with commercial sequencing by Eurofins Genomics (Louisville, KY, USA). Sequences were edited using 4PEAKS (Nucleobytes, The Netherlands). The El Cordón specimen is currently stored in silica gel at the University of Alabama at Birmingham (USA).

We aligned all the mitochondrial and plastid sequences using MUSCLE (Edgar 2004) in SEAVIEW ver. 4.6 (Galtier *et al.*, 1996, Gouy *et al.*, 2010) with default parameters. The *cox*1 (KY056219, this study) and *rbc*L sequences (KY056221, this study) matched sequences from Mexico, Hawai'i, South Korea and Japan (*cox*1: KC113591-94, García-Rodríguez *et al.*, 2013; EF434921-22, Yang *et al*., 2008; *rbc*L: KC113597-99, García-Rodríguez *et al*., 2013; EF434942,45, Kim *et al.*, 2008; HQ886637-38, Muangmai, *unpubl. data*). The *cox*1 alignment, when trimmed, was 785 bp and the El Cordón specimen was 100% similar to the two sequences in GenBank from Mexico (KC113591-92) and 99% similar to the Hawai'ian and Northwestern Pacific sequences (KC113593-94, EF434921-22). The *rbc*L alignment, when trimmed, was 936 base pairs and the El Cordón sequence was 100% similar to two of the three Mexican specimens (KC113597-98) and the Hawai'ian specimen (EF434945), whereas with the other Mexican sample, there was 1 base pair difference

(KC113599). Both Mexican and Hawai'ian sequences were 99% similar to the other Northwest Pacific sequences (EF434942, HQ886637-38).

In contrast, the *cox*2-3 spacer sequence (KY056220, this study) matched sequences identified as *Gracilaria gigas* Harvey (type locality: Shimoda, Shizuoka Prefecture) from two sites in Japan and reported in Terada & Shimada (2005; AB193451-52). *G. gigas* is an accepted species reported from the Northwest Pacific and southwestern and southeastern Asia (Guiry & Guiry, 2016). No studies, to our knowledge, have investigated the type specimens of *G. parvispora* and *G. gigas* with molecular methods to obtain authentic sequence data or compared contemporary specimens of each species. *G. parvispora* was first described from a 1970 specimen from Hawai'i (Abbot, 1985), whereas *G. gigas* was first described in 1860 from a Japanese specimen (Masuda *et al*., 1995). If these two species are conspecific, the correct name for the species currently known as *G. parvispora* should be *G. gigas* by the nomenclatural law of priority. However, as the *cox*1 and *rbc*L sequences match GenBank specimens identified as *G. parvispora* throughout its natural and introduced range (see above) and because there is little current information about *G. gigas*, it is likely that the El Cordón specimen is the same as others currently identified as *G. parvispora.* The results of this study suggest that the specimens cited by Terada & Shimada (2005) as *G. gigas* were misidentifications.

Though we only sampled a single thallus, the species previously studied in Vergara-Rodarte *et al*., (2010, 2016) is *G. parvispora*. Zertuche-Gonzalez (1993) mentioned a bed of *Gracilaria pacifica* located in Laguna de San Ignacio, which was exploited since 1993 for the agar production. The gracilarioid bed was approximately 60 hectares and could produce a total of 900 dry tons per year, from which 20 tons were exported Japan. Further studies found that *G. pacifica* was the most abundant species and was present along all year at Laguna de San Ignacio (Nuñez-Lopez *et al*., 1998, Nuñez-Lopez & Casas-Valdez, 1998). Riosmena-Rodríguez *et al*., (2009) and Vergara-Rodarte *et al*., (2010) reported the most abundant species in Laguna de San Ignacio was *G. vermiculophylla* and not *G. pacifica* based on morphological characteristics. Moreover, the most abundant gracilarioid seaweed, thought to be *G. vermiculophylla*, formed monospecific bed with a maximum biomass in spring (1,004 wet tons, Vergara-Rodarte *et al*., 2010, 2016). Though we cannot dismiss the co-occurrence of other taxa, especially *G. vermiculophylla* (see García-Rodríguez *et al*., 2013), the dominant species previously studied in Laguna de San Ignacio and genotyped in this study is *G. parvispora*.

This new record adds another location along the Baja California peninsula where this species is abundant (Fig. 1). In Laguna San Ignacio, tetrasporic plants were the most common, followed by vegetative plants, though it is not known if these are sterile (Vergara-Rodarte *et al*., 2016). García-Rodríguez *et al*., (2013) sampled both tetrasporophytes and female gametophytes at the sites in northwest Mexico where they first recorded *G. parvispora*. Recently, Krueger-Hadfield *et al*., (2016a) found similar patterns in the congeneric and often associated introduced and invasive species, *G. vermiculophylla*. In free-floating populations, *G. vermiculophylla* plants were overwhelmingly tetrasporophytic (Krueger-Hadfield *et al*., 2016a). However, there were some sites in the introduced range with abundant hard substratum in which both tetrasporophytic and gametophytic plants were sampled (Krueger-Hadfield *et al*., 2016a). Due to the potential of *G. parvispora* as a source of agar, more detailed studies of the invasion history and extensive surveys to determine its current distribution and impacts on the recipient ecosystems.

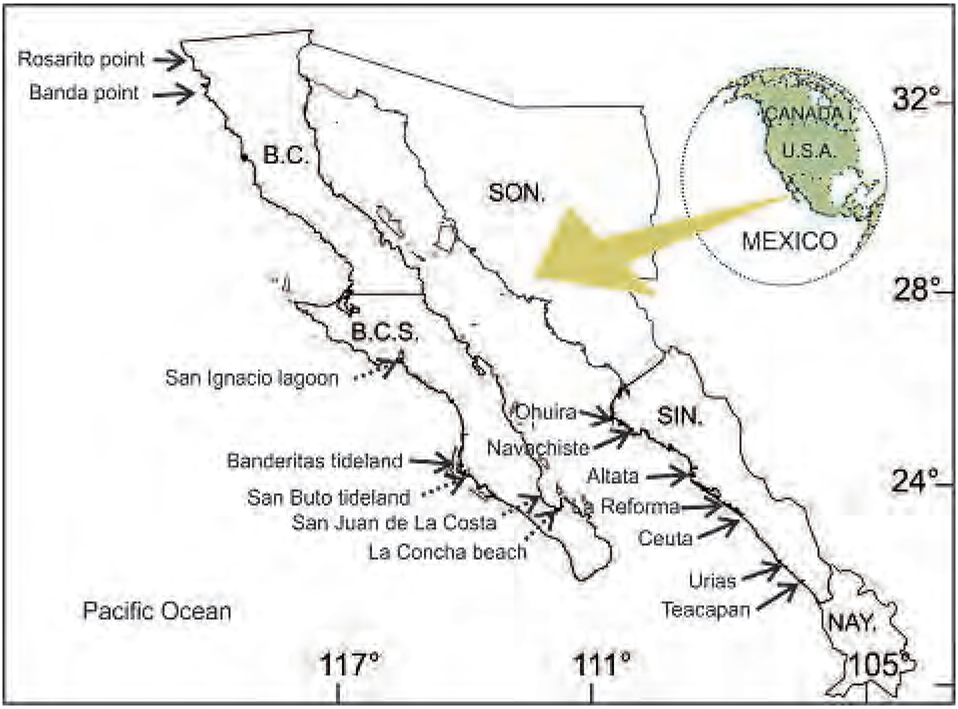

Fig. 1. The known distribution of *Gracilaria parvispora* (dashed arrow) and *Gracilaria vermicullophylla* (solid arrows) along the Mexican coast based on previous studies (see review in García-Rodríguez *et al.*, 2013). Laguna de San Ignacio is a new record for *G. parvispora*.

Acknowledgements. Funding was provided by the University of Alabama at Birmingham to SA Krueger-Hadfield and IPN-COFAA fellowship granted under the Program of Exclusivity (Beca de Exclusividad) and the EDI program (Estímulo al Desempeño de los Investigadores del IPN) to G. Hernández-Carmona. We thank K.A. Miller, L. Le Gall and an anonymous reviewer for suggestions that greatly improved this manuscript.

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