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Short Communication

First confirmed reports of the rhizostome jellyfish *Mastigias* (Cnidaria: Rhizostomeae) in the Atlantic basin

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Abstract

The rhizostome jellyfish *Mastigias* (Agassiz, 1862), native to the western and central Indo-Pacific, is recorded from the western Atlantic, in a marine lake in Puerto Rico (Laguna Joyuda) and a working quarry on No Name Key, Florida (USA) in the Florida Keys. The single individual from Laguna Joyuda, collected in 2002, was previously misidentified as *Phyllorhiza* (Agassiz, 1862) and *Mastigias* has not since been recorded there, while a large population of *Mastigias* was observed in No Name Key in 2009 and 2010 and may have been present for decades. Identification as *Mastigias* for both sampling sites was confirmed by molecular systematic analysis of mitochondrial cytochrome c oxidase I (COI) and ribosomal 16S (16S). Sequences from both molecular markers were identical for all individuals sampled from Puerto Rico and the Florida Keys. Phylogenetic analysis of COI indicated that the introduced *Mastigias* were most closely related to *Mastigias* sp. 1 (Dawson, 2005) from Kakaban, Indonesia (Kimura 2-parameter sequence divergence = 1.1%) and distantly related to *Mastigias papua* (Lesson, 1830) from Palau (6.4-7.2%) and *Mastigias* sp. 2 (Dawson, 2005) from Papua New Guinea (9.8%). Therefore, the source region may lie somewhere in the central Indo-Malayan region, though determining an exact source region is not possible at this time. While the invasion vector is unclear, possible mechanisms include commercial shipping, mobile oil platforms and live rock transport. These are the first two confirmed records of *Mastigias* in the Atlantic basin and the second confirmed identification of the genus occurring outside of the native range, after Hawaii.

Key words: gelatinous zooplankton, medusa, marine lakes, rhizostome, introduced species, molecular systematics, cytochrome c oxidase subunit I.

The jellyfish genus *Mastigias* (Scyphozoa: Rhizostomeae) is native to the western and central Indo-Pacific, from Australia to Japan, and Micronesia to the Indian Ocean (Kramp 1961). In the native range, these jellyfish occupy coastal and lagoonal waters, but are most notably found in marine lakes, where they can form famously large aggregations that are important tourist attractions (Dawson et al. 2001). Investigations of *Mastigias* populations from marine lakes in Palau have revealed important information regarding evolution within the species *Mastigias papua*, indicating rapid and profound ecological and behavioral adaptation that accompanied their isolation in newly formed marine lakes (Dawson and Hamner 2003; Dawson and Hamner 2005), as well as the formation of multiple subspecies of *Mastigias* (Dawson 2005a).

Study of the spread of *Mastigias* outside of the Indo-Pacific has been hindered by historical confusion in the literature differentiating between *Mastigias* and *Phyllorhiza*, another genus in Family Mastigiidae. Although the generic definitions of these two genera were stabilized by Stiasny (1924), some confusion has remained. *Phyllorhiza* is one of the most effective introduced scyphozoans on record, having invaded every major non-native marine water body except the Arctic and Antarctic (reviewed in Bolton and Graham 2004). However, jellyfish from several of these invaded regions were originally identified as *Mastigias* and later determined to have been misidentifications of *Phyllorhiza*. For instance, Moreira (1961) described *Mastigias scintillae* (Moreira, 1961) from a population in Brazilian waters that subsequently disappeared. However, it is generally accepted that *Mastigias scintillae* was actually *Phyllorhiza*, like the recently reappeared Brazilian *Phyllorhiza* populations (Mianzan and Cornelius 1999; Silveira and Cornelius 2000;

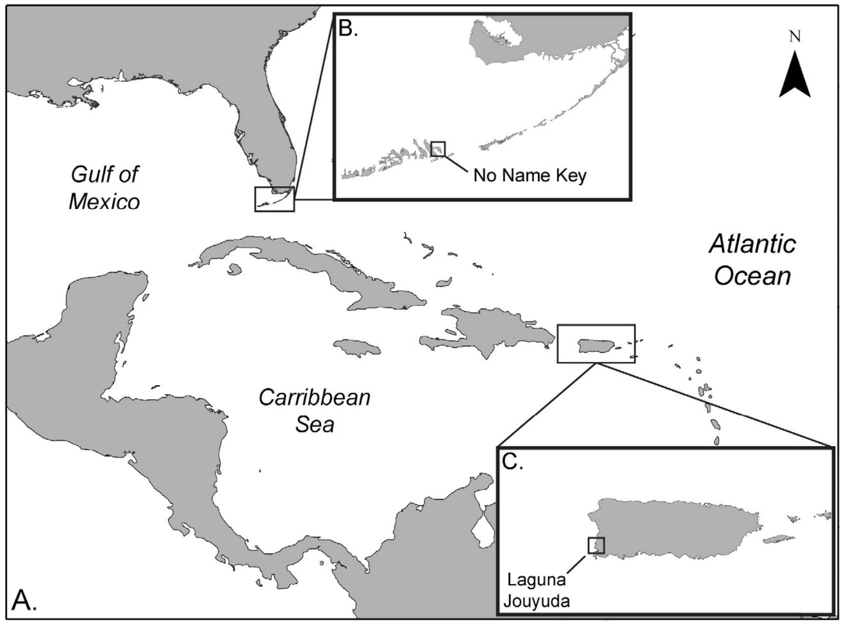

Figure 1. A) Map of the Caribbean Sea detailing location of introduced *Mastigias* populations; B) Inset showing location of No Name Key, Florida (USA). The working quarry where the population is located is on private property at approximately 24°41'41.92″N 81°19'47.01″W; C) Inset showing the location of Laguna Joyuda on the west coast of Puerto Rico. The lake is located at approximately 18°07'21.47″N 67°10'46.78″W.

Migotto et al. 2002). Likewise, *Mastigias albipunctatus* (Stiasny, 1920) identified from Jamaican waters (Vannucci 1964) was also a likely misidentification of *Phyllorhiza* (Cutress 1971), which was recently photographed in Jamaica (L. Brotz, pers. comm.) and is found elsewhere in the Caribbean in Puerto Rico (Cutress 1971; Garcia 1990; Garcia and Durbin 1993). However, recent observations confirm that both *Phyllorhiza* and *Mastigias* occur in Hawaiian coastal waters (Eldredge and Smith 2001).

During a morphology-based study of world-wide populations of *Phyllorhiza* designed to indicate source region(s) of invaders, Bolton and Graham (2004) examined *Phyllorhiza* medusae from six worldwide populations, including Laguna Joyuda, Puerto Rico (Figure 1). While ten medusae from the lagoon were examined morphologically, the paper included a photo-graph of a medusa from Laguna Joyuda not used in the morphological analyses (Figure 2) that resembled *Mastigias* more so than *Phyllorhiza*. A more recent study investigating the molecular phylogeography of *Phyllorhiza* examined tissue samples from the specimens studied in Bolton and Graham (2004), with all genetic data indicating that these specimens belonged to the genus *Phyllorhiza* (Bayha, unpubl. data). However, questions remained regarding the correct species designation of the animal pictured in Bolton and Graham (2004) (Figure 2), which, while not previously examined morphologically or molecularly, was preserved in ethanol after collection in 2002 and stored at -20^{o}C at the Dauphin Island Sea Lab (Dauphin Island, Alabama, USA).

In October of 2009, we were made aware of a large population of rhizostome jellyfish in a working, privately owned quarry on No Name Key, Florida (U.S.A.) in the Florida Keys (Figure 1). Initial photographs sent to us suggested these jellyfish were *Mastigias*. In late October 2009, we collected and photographed six (6) individuals from the quarry, preserving oral arm and gonad tissue in ethanol (for DNA analysis) and whole animals in 10% formalin (for morphology). Morphological indications were that all jellyfish encountered were *Mastigias* according to previous taxonomic definitions (Stiasny 1924; Kramp 1961) (Figure 3). A later excursion to the site in July 2010 observed a population of similar size to the one in 2009, suggesting that the population persists from year to year. According to quarry personnel, this population has been present in the quarry for as many as 20 years. (V. Rocho, pers. comm.). Two individuals, preserved in 10%

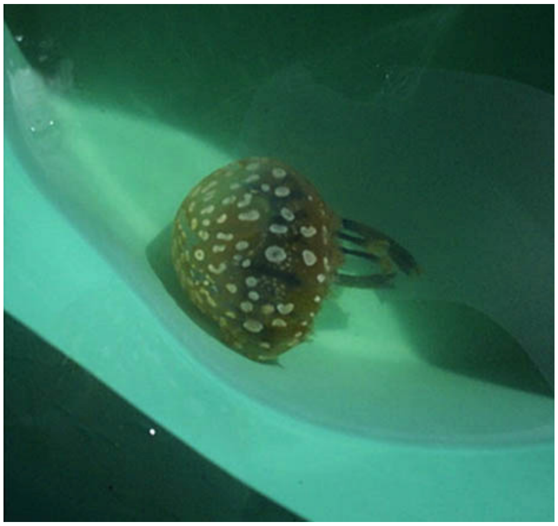

Figure 2. *Mastigias* sp. 1 collected from Laguna Joyuda, Puerto Rico in July2002. Bell diameter is approximately 4 cm. Photograph by T. Bolton.

Figure 3. *Mastigias* sp. 1 collected from No Name Key, Florida (USA) in October 2009. Bell diameter is approximately 6 cm. Photograph by M. Miller.

formalin, have been deposited at the National Museum of Natural History at the Smithsonian Institution (USNM 1156086).

Since the use of molecular genetic techniques has been instrumental in determining species boundaries in scyphozoan jellies (Dawson and Jacobs 2001; Holland et al. 2004; Dawson 2005b; Bayha and Dawson 2010) as well as correcting morphological misidentifications of gelatinous zooplankton from past publications (Gorokhova et al. 2009), we sequenced mitochondrial *COI* and *16S* for three individuals from No Name Key and one individual from Laguna Joyuda. DNA was extracted from each sample using a standard CTAB protocol (Winnepenninckx et al. 1993). A 658 base pair region of *COI* was amplified using primers LCO1490 and HCO2198 (Folmer et al. 1994), under the following conditions: 94°C for 120 seconds (s), 38 cycles of 94°C for 30 s, 48°C for 45 s and 72°C for 75 s, followed by 72°C for 10 mins (minutes) and refrigeration at 4°C. A 585 base pair region of *16S* was amplified using primers *16S*-L (Ender and Schierwater 2003) and Aa_H*16S*_15141 (Bayha and Dawson 2010), and conditions consisted of 94°C for 120 s, 38 cycles of 94°C for 30 s, 52°C for 45 s and 72°C for 60s, followed by 72°C for 10 mins and refrigeration at 4°C. PCR success was verified by running PCR products out on a 2% agarose gel. PCR products were purified and then bidirectionally cycle-sequenced by Beckman-Coulter Genomics (Beverly, MA) using the PCR primers above. Individual sequences were assembled using Lasergene Seqman Pro v. 8.1.5 (DNAStar, Inc.) and identities of the genetic regions were confirmed using BLASTn or BLASTx (Altschul et al. 1997). Since no *Mastigias 16S* sequence occurs in NCBI GenBank at present, we compared our sequences to *16S* sequence from *Mastigias* sp. (unknown geographic origin) collected from the Seto Marine Laboratory (Honshu, Japan) and contributed by Allen Collins (National Systematics Lab of NOAA's Fisheries Service and Smithsonian National Museum of Natural History). DNA sequences have been deposited in NCBI GenBank (Accession numbers JN215543-JN215551).

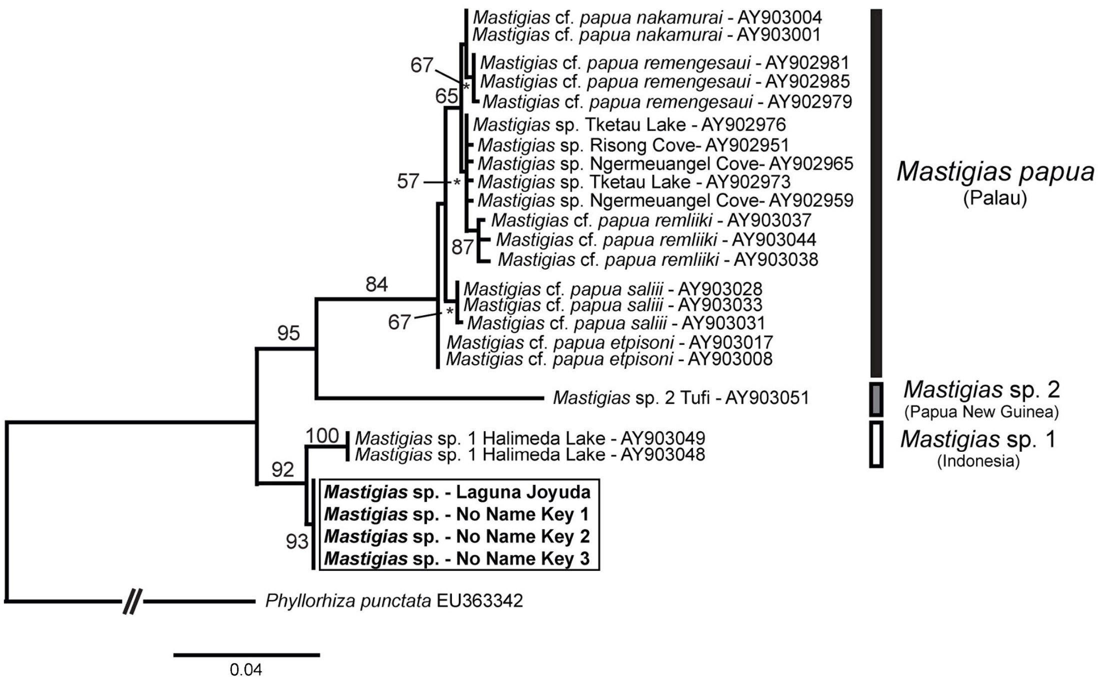

Figure 4. Phylogenetic relationships between introduced *Mastigias* sp. and native *Mastigias* spp. reconstructed using maximum likelihood analysis of *COI* with substitution model HKY+I. The tree is rooted with COI from *Phyllorhiza punctata* (NCBI Accession #EU363342) and the broken branch leading to the outgroup has been shortened by 50%. Numbers adjacent to branches show bootstrap support if >50% based on 500 bootstraps. Our phylogenetic analyses indicate that the invasive *Mastigias* in the Florida Keys and Puerto Rico are likely the same species as those found in Indonesia (*Mastigias* sp. 1).

All DNA sequences obtained from Laguna Joyuda and No Name Key were identical for *COI* and *16S* respectively and the data are consistent with all individuals belonging to the genus *Mastigias*. Sequence divergences (Kimura 2-parameter distance) between invasive jellyfish and established *Mastigias* spp. averaged 6.4% (1.1-9.8%) for *COI* and 0.5% for *16S*. Maximum Likelihood analyses of these sequences along with a subset of *Mastigias* sequences published in NCBI GenBank representing all described subspecies of *Mastigias papua* from Palau, as well as *Mastigias* spp. from Indonesia and Papua New Guinea (Dawson 2005a; Dawson and Hamner 2005), was carried out using Garli v. 0.951 under the HKY+I model as determined using jModeltest v. 0.11 (Posada 2008) under the Bayesian Information Criterion (BIC). The phylogeny indicated a close relationship between *Mastigias* from Laguna Joyuda and No Name Key and *Mastigias* sp. 1 from Kakaban, Indonesia (sequence divergence = 1.1%), but a distant relationship to *Mastigias papua* found in Palau (sequence divergence = 6.4-7.2%; average=6.8%) and *Mastigias* sp. 2 found in Papua New Guinea (sequence divergence = 9.8%) (Figure 4). None of the introduced animals were genetically identical to native animals published in NCBI GenBank. This may indicate that the source region of the invasive animals is somewhere in the central Indo-Malayan region (though not Kakaban, Indonesia), but not in Papua New Guinea or Micronesia (Palau), unless multiple species overlap in some of these regions. While we confirm the presence of *Mastigias* sp. 1 in the Caribbean and Gulf of Mexico, the specific source cannot be determined given the small geographic sampling present in the NCBI GenBank database at this time for *Mastigias*. An additional study incorporating a larger number of native populations may be more successful in determining the exact source region(s) of the invasive *Mastigias*.

Our data bring to a close some of the uncertainty surrounding the *Mastigias*-like picture from Bolton and Graham (2004) by showing that the individual is genetically *Mastigias*, but questions remain about how prevalent *Mastigias* is in Laguna Joyuda and whether or not the two species co-occur on a regular basis. While the 2002 survey produced both *Phyllorhiza* and *Mastigias*, confirmed here by molecular data, a survey in 2010 only produced *Phyllorhiza* and no co-occurrence of the two species (Bayha, unpubl. data). Strangely, while the 10 medusae from Laguna Joyuda examined morphologically in Bolton and Graham (2004) showed some characters consistent with *Mastigias* (large spots, etc.), all of them were genetically *Phyllorhiza* for both mitochondrial (cytochrome oxidase subunit III [*COIII*] and *16S*) and nuclear (internal transcribed spacer region I [*ITS-1*]) regions (Bayha, unpubl. data). The lack of any highly divergent *ITS-1*sequences within a *Phyllorhiza* individual for the Laguna Joyuda *Phyllorhiza* (2002 and 2010) (Bayha, unpubl. data) did not support hybridization between *Phyllorhiza* and *Mastigias* as a factor.

While we have confirmed the presence of *Mastigias* in the Florida Keys and Puerto Rico, possibly originating from the central Indo-Malayan region, how the jellyfish arrived in the Caribbean/Gulf of Mexico remains unclear. Laguna Joyuda is open to the ocean to the south and while the quarry on No Name Key does not have a direct connection to open water, it shows definite indications of intermittent connection, either through deep cracks or through overwash, since the quarry has an abundant population of marine phytoplankton, copepods and even fish species (W.M. Graham, pers. obs.). It is possible that one or two of the regions were colonized by benthic scyphistomae from ocean-going vessels as is hypothesized for *Phyllorhiza* (Larson and Arneson 1990; Graham et al. 2003), via movable oil platforms (Yeo et al. 2010) or artificial reef materials (Sheehy and Vik 2010), and since all animals are genetically identical for *COI* and *16S*, one population may have seeded the other. Another possibility is via the worldwide trade in “live rock” for aquariums, in which rock (and all attached biota) is removed from the edges of reef areas, primarily in the Indo-Pacific, and transported worldwide to be placed in aquaria (Wabnitz et al. 2003). Bolton and Graham (2006) showed that live rock imported to the U.S. from the Indo-Pacific (possibly Indonesia or Fiji) contained viable polyps of the jellyfish *Cassiopea* sp., which actively asexually reproduced ephyrae. Therefore, it is possible that *Mastigias* may have originally arrived in Atlantic waters as viable polyps on live rock.

While these new invasive *Mastigias* populations occur in enclosed or semi-enclosed marine lakes and the effects of these new populations are likely local in nature, *Mastigias* does occur in other coastal regions (lagoons, bays, harbors, etc.) in its native range (Dawson and Hamner 2003), meaning it could spread to other coastal regions of the Atlantic. Hence, the establishment of *Mastigias* in the Atlantic basin could mark the first signs of a worldwide spread of *Mastigias* either by natural transport from donor regions or by anthropogenic means (shipping, aquarium trade, etc.), similar to that of another mastigiid jellyfish *Phyllorhiza* (reviewed in Bolton and Graham 2004), which has had concomitant ecological and economic effects on recipient regions (Graham et al. 2003) like other invasive gelatinous zooplankton (reviewed in Graham and Bayha 2007). While the specific mechanisms bringing *Mastigias* to the Atlantic basin are not established, its presence in the Caribbean and Gulf of Mexico is clear and future studies should be on the watch for this jellyfish throughout the region.

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