

Rapid Communication**First record of *Scinaia cf. johnstoniae* (Nemaliales, Rhodophyta) in Gwaii Haanas, British Columbia, Canada**

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OPEN ACCESS**Abstract**

In July 2019, three unusual red algal specimens field identified as *Scinaia interrupta* were found in Gwaii Haanas, a marine conservation area and heritage site in the Haida Gwaii archipelago, British Columbia. As one of the authors (GWS) has collected relatively extensively in these waters, the collection of these distinctive specimens was unexpected. The DNA barcode COI-5P was used to assess the field identification and the three individuals were closely allied (0–1 bp difference; 0.15%) in a genetic group that was a close sister (7–9 bp difference; 1.1–1.4%) to an earlier collection from southern British Columbia, and a collection from near the type locality in Brittany, France for specimens assigned to *S. interrupta*. This observation is consistent with separate introduction events for these two populations in British Columbia, although the story may be more complex. Anatomical observations in the laboratory revealed male structures consistent with the Pacific species *Scinaia johnstoniae* for which there is regrettably no comparative molecular data. Owing to the typically conservative nature of red algal reproductive features, it would be unusual for two such closely related genetic groups to have such divergent male reproduction. For now, we can report on the discovery of a novel *Scinaia* sp. in Gwaii Haanas tentatively assigned to *Scinaia johnstoniae*.

Key words: *Scinaia interrupta*, introduced species, Haida Gwaii, COI-5P**Introduction**

Scinaia interrupta (A.P. de Candolle) M.J. Wynne 1989, an alga described from Brittany, France is reportedly widely distributed in European waters extending from Ireland to the Azores, Canary Islands, and into the Mediterranean, as well as along the Moroccan Atlantic coast (Guiry and Guiry 2020). It is not generally a species that comes to mind when discussing invasive or introduced seaweeds, but it was reported earlier from more southern waters in British Columbia (Le Gall and Saunders 2010). That report was based on a single subtidal (10 m) specimen that was collected as a thin, red algal crust. It was possibly the filamentous sporophytic stage of *S. interrupta*, which is characterized by an alternation of heteromorphic generations (León-Cisneros et al. 2011). However, the result may also have resulted from PCR contamination in the laboratory; a possibility buoyed

by the lack of additional collections for either stage in the life history of this species over the subsequent decade of collecting in British Columbia.

Around the same time as the publication of Le Gall and Saunders (2010), León-Cisneros et al. (2009) reported *Scinaia interrupta* from the Gulf of California, Mexico. Unfortunately, their identification was not confirmed with molecular data contributing to the uncertainty of the presence of this species in Pacific waters. Morphologically their collections were certainly a possible match. Surprisingly, these authors did not discuss the presumably unexpected encounter of this species in their Mexican flora. Nonetheless, their discovery combined with that of Le Gall and Saunders (2010) only intensified our mostly unsuccessful efforts over the intervening decade to find additional collections of this species in British Columbia.

On a recent exploratory dive in Haida Gwaii in 2019, however, we encountered three individuals identified as the gametophyte phase of *S. interrupta*. Molecular work revealed the COI-5P (5' end of the cytochrome *c* oxidase subunit I gene) sequences also matched *S. interrupta* but conflicted with further anatomical observations suggestive of the related *S. johnstoniae* Setchell 1914. Clearly further study is needed, but we can for now report on the recent arrival of an additional species of *Scinaia* to Gwaii Haanas in northern British Columbia.

Materials and methods

Plants were collected (20 July 2019) during a single 6 m dive at one site in the Gwaii Haanas National Park Reserve, National Marine Conservation Area Reserve, and Haida Heritage Site (Figure 1). Specimens were pressed as vouchers (GWS045682, GWS045683, GWS045705; housed in the Connell Memorial Herbarium, UNB) with subsamples of each individual dried in silica gel for subsequent DNA extraction (Saunders and McDevit 2012) and amplification of the DNA barcode marker COI-5P following PCR profiles outlined in Saunders and Moore (2013). Amplified product was sent to Génome Québec for bidirectional sequencing on an Applied Biosystem 3730xl DNA Analyzer. Raw data were edited using Geneious Prime 2020.1.1 (Biomatters Inc., San Diego) to a final length of 651–664 bp. Edited sequences were deposited in GenBank (MT358271, MT358272, MT358270; respectively) and searched through BOLD (Barcode of Life Data System; Ratnasingham and Hebert 2007) and GenBank to determine identifications.

Algal tissue was rehydrated in 5% formaldehyde in seawater and then sectioned with a freezing microtome (CM18250, Leica, Heidelberg, Germany) for anatomical observations with the Leica microscope (CTR5000).

Results

Based on the DNA barcode marker COI-5P, the three 2019 specimens field-identified as *Scinaia interrupta* (Figure 2) were closely allied (only 0–1 bp or 0–0.15% divergent over the 664 bp of the marker). Interestingly, they were



Figure 1. Map of Haida Gwaii with a close-up of the dive site in the channel between Faraday Is. and Murchison Is. (52.59687N; 131.47513W), Gwaii Haanas National Park Reserve, National Marine Conservation Area Reserve, and Haida Heritage Site. ArcGIS Online © 2020.



Figure 2. A gametophyte of *Scinia cf. johnstoniae* collected from the subtidal (6 m) on cobble in the channel between Faraday Is. and Murchison Is., Gwaii Haanas (GWS045682). Coin included for scale (2.5 cm diameter). Photo by Gary Saunders.

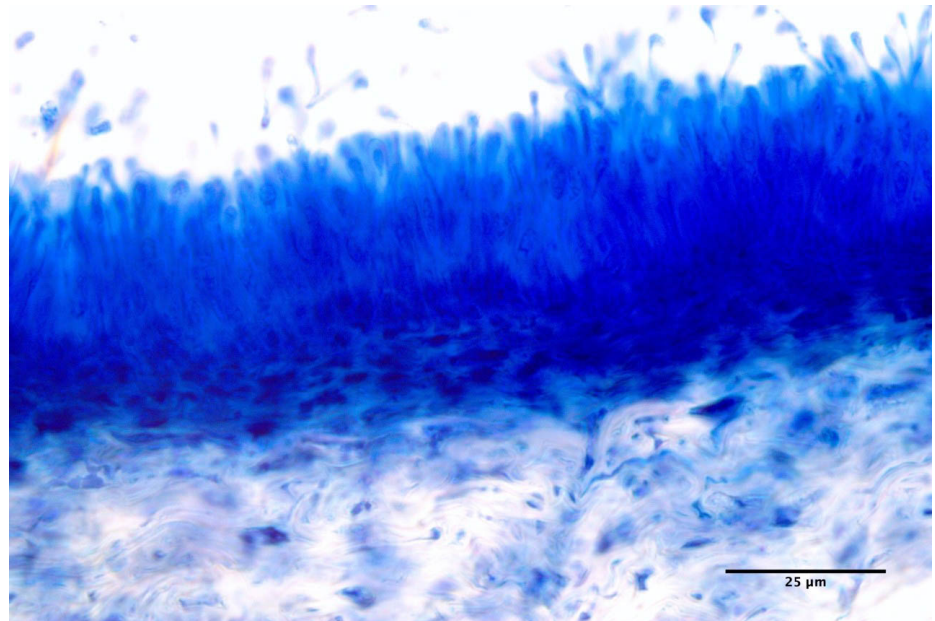


Figure 3. Spermatangia form in extensive sori over the branch apices (GWS045683). Photo by Gary Saunders.

1.1–1.4% divergent (7–9 bp) from a collection from near the type locality in France (HQ603259) and an earlier collection from southern British Columbia (HQ544543), while these latter two collections were themselves only 0.15% divergent. The former level of divergence can represent divergent populations of a single species or two closely related sibling species. Consequently, anatomical observations were completed. Fortunately, one of our specimens was male and revealed spermatangia distributed in extensive sori capping the branch tips consistent with the species *S. johnstoniae* rather than *S. interrupta* (Figure 3).

Discussion

After a decade of opportunistic searching (collection trips in 2011–15, 2018–19), it was a surprise to collect these three individuals, which were identified in the field as *Scinaia interrupta*. A highly distinctive morphology relative to all other seaweeds in Haida Gwaii including the expected *Scinaia confusa* (Setchell) Huisman 1985, it is not something that would have gone unnoticed. It seems probable that this is a recent introduction to the Haida Gwaii flora. Subsequent molecular work reported here were consistent with this identification, at least in part.

The Haida Gwaii collections were 1.1–1.4% divergent from an earlier collection from southern British Columbia (putative sporophyte, GWS019633, Bamfield, subtidal 10 m on a shell). Divergence values for COI-5P in the range between 1–2% fall in a gray zone (Saunders 2005, 2008) between being distinct populations or distinct species (e.g. *Mazzaella splendens* (Setchell & N.L.Gardner) Fredericq, 1994, versus *M. dewreedei* G.W. Saunders & K.R. Millar, 2014 and *M. linearis* (Setchell & N.L. Gardner) Fredericq, 1993; Saunders and Millar 2014). Thus, these collections are

most likely recent introductions to Haida Gwaii, but are unlikely to be linked to the presumptive introduction event that led to the earlier putative single collection in Bamfield (Le Gall and Saunders 2010). Scenarios to explain the presence of these individuals in Haida Gwaii consequently must consider two separate introductions while addressing the identification uncertainty. Four plausible hypotheses are foremost in our sights for testing.

Hypothesis 1. If these individuals are a distinct population of *Scinaia interrupta*, which is consistent with the molecular data, then the two introduction events were separate introductions directly from different populations in the European flora (presumably through shipping or the movement of marine infrastructure, water or biological resources). For example, our collections of *Rhodymenia delicatula* P.J.L. Dangeard 1949 from an introduced population at Cape Cod, Massachusetts were 1.4–1.5% divergent in COI-5P from an Irish specimen raising the possibility that the northwest Atlantic population was refugial (unexpectedly common in the NW Atlantic flora; see Bringloe and Saunders 2018) rather than introduced. However, data from southern Europe eventually were submitted to BOLD (Le Gall *unpublished*) that were only 0.5–0.6% divergent from our Cape Cod collections raising the possibility again of an introduction, but from Mediterranean waters (G. Saunders *unpublished*). In short, more comprehensive sampling in Europe could shed light on this hypothesis.

Hypothesis 2. The Mexican population of *Scinaia interrupta* may also be of recent origin, which may indicate an introduction to that flora, although this was not speculated by the authors (León-Cisneros et al. 2009). The species may have subsequently spread northward to California since its earlier discovery. Thereafter, this species could become entangled in kelp rafts, facilitating transport north on the kelp conveyor (Saunders 2014) possibly explaining the recent arrival in Haida Gwaii. It would mean that the Mexican specimens will genetically match the more geographically remote Haida Gwaii rather than Bamfield population, which is testable.

However, based on observations in León-Cisneros et al. (2009), the spermantangial features observed for our genetic group (Figure 3) would assign it to *S. johnstoniae* and not *S. interrupta*. This possibility is not inconsistent with the molecular data assuming these are very closely related sibling species. However, as reproductive features are generally considered to be conservative in red algae (Saunders and Hommersand 2004), the differences in spermantangial features and carposporophyte structure reported in León-Cisneros et al. (2009) are contrary to a sibling relationship between these two species. Although *S. johnstoniae* is reported from warm water locations (León-Cisneros et al. 2009), its full range into cooler waters of Asia and California is unknown. Nonetheless, warming temperatures in the waters around Haida Gwaii (Freeland 1990) could possibly explain the survival of this species in this area following a recent introduction event. This hypothesis should not be rejected without further

study, notably molecular data are needed from toptype collections of *S. johnstoniae*. For now, if we assume this genetic group is assignable to *S. johnstoniae*, then two additional hypotheses are plausible.

Hypothesis 3. *Scinaia johnstoniae* is reported in the Japanese flora (as *S. tokidae* Kajimura, 1988; Lee and Ko 2006; León-Cisneros et al. 2009). This species could thus have made its way to the Northeast Pacific (Haida Gwaii in the present case) on tsunami debris as has been reported for other algal species (Hansen et al. 2018). Again, this is a testable hypothesis that will require extensive collection of individuals with the *S. johnstoniae*-type morphology in Japan. This appears to be the most parsimonious hypothesis.

Hypothesis 4. This final hypothesis does not include a recent anthropogenic vector. *Scinaia johnstoniae* is reported in the California flora (see León-Cisneros et al. 2009). A direct, albeit recent, transfer north from California on the kelp conveyor is a possibility (Saunders 2014).

The four hypotheses presented above are all testable hypotheses awaiting more collections with corresponding DNA barcode data. Regrettably, comparative molecular data could not be found in BOLD or GenBank for *Scinaia johnstoniae*. Molecular data for *S. johnstoniae* are necessary to confirm the taxonomic conclusions of León-Cisneros et al. (2009), as well as the tentative assignment of our specimens to *S. johnstoniae*. With a definitive identification in hand, molecular data for specimens from California and Japan, as well as better sampling from Europe and Mexico will be necessary to support one of the hypotheses presented here. Only the last hypothesis could be considered a natural migration of this novel *Scinaia* sp. to Haida Gwaii, whereas as all other hypotheses indicate some degree of anthropogenic involvement. Regardless, this discovery represents a species new to Haida Gwaii and thus worthy of further study.

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