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5 **The marine sponge *Hymeniacidon perlevis* is a globally-distributed invasive species**
6

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16 **Abstract**
17 In Elkhorn Slough, a tidal estuary draining into Monterey Bay, California, the intertidal is
18 occupied by a conspicuous orange sponge known by the name *Hymeniacidon sinapium*.
19 This same species is found in the rocky intertidal zone of the outer coast of California,
20 and is described herein from subtidal kelp forests of Southern California. Farther afield,
21 morphologically and ecologically indistinguishable sponges are common in estuaries and
22 intertidal areas in Asia, Europe, South America, and Africa. Here I use morphological,
23 ecological, and genetic data to show that these sponges are all members of the same
24 globally-distributed species, which should be known by the senior synonym *H. perlevis*.
25 Though previous authors have remarked upon the morphological, ecological, and/or
26 genetic similarity of various distant populations, the true scope of this sponge's
27 distribution appears to be unrecognized or unacknowledged in the literature. Limited
28 larval dispersal, historically documented range expansion, and low genetic variation all
29 support a hypothesis that this sponge has achieved its extraordinary range via human-
30 mediated dispersal, making it the most widely-distributed invasive sponge known to date.
31

32 **Keywords**
33 Porifera, sponges, invasive species, kelp forest, intertidal, estuaries
34

Introduction

In coastal marine ecosystems, filter-feeding marine invertebrates are among the most important invasive species in terms of species diversity, biomass, and ecological impacts (Ruiz et al. 2000; Bax et al. 2003; Byrnes and Stachowicz 2009). Sponges (phylum Porifera) are a diverse group of filter-feeding organisms that are found in all marine environments. They provide unique ecosystems services (and potential disruptions) because they preferentially consume the smaller size fractions of the plankton, such as viral and bacterial plankton (Reiswig 1971; Maldonado et al. 2012; Welsh et al. 2020). They can also have major effects on nutrient cycling, as some sponges convert dissolved nutrients into particulate matter available to other animals (de Goeij et al. 2013).

Our understanding of invasive sponges has been limited by an incomplete taxonomy. Sponges have simpler morphologies than most animals, confounding traditional classification schemes (Morrow and Cárdenas 2015). Many species were initially described as having a wide geographic range, but in recent decades these taxa have been recognized as clades comprised of multiple species with similar morphologies (Knowlton 1993; Xavier et al. 2010). This is consistent with what is known about larval dispersal in sponges. All known sponge larvae are lecithotrophic, meaning that they have no ability to feed until they settle and develop into juveniles (Maldonado 2006). They have a short planktonic stage, lasting from minutes to a few days (Maldonado 2006). Some sponges, however, do seem to have broad geographic ranges, and this is likely due to human-mediated transport. Carballo et al. (2013) list seven species thought to have recent range expansions, including two that have moved between the Pacific and Atlantic basins. Some of these species are likely to have been accidentally introduced with aquaculture (Henkel and Janussen 2011; Fuller and Hughey 2013). Trawling, hull-fouling, and other activities also likely play a role (Carballo et al. 2013).

In the current work, I describe what appears to be the most common and widely distributed invasive sponge known to date. Genetic and morphological data support a distribution that includes Europe, the Atlantic coasts of North and South America, the Pacific coast of North America, and Asia. Morphological data suggest it is also present in New Zealand, Southwest Africa, and the Pacific coast of South America, but genetic data are not yet available from these populations. In much of this range, it is among the most common sponges in multiple habitats. In Europe, this species is known as *Hymeniacidon perlevis* (Montagu 1814). The range of *H. perlevis* was already thought to be substantial: from Norway in the North to the Macronesian Islands off Africa in the South (Erpenbeck and Van Soest 2002). Within this range it is found in diverse habitats, including both the intertidal and the subtidal zones, and it can grow buried in sediment or on hard substrate (Erpenbeck and Van Soest 2002). It is often abundant in these habitats, and is considered to be one of the most common sponges in Europe (Erpenbeck and Van Soest 2002). It has been described by other taxonomists as also occurring in New Zealand (Bergquist 1961, 1970) and as the most abundant intertidal sponge in Western South Africa (Samaai and Gibbons 2005), but these records were rejected from the consensus view (Van Soest et al. 2020a), probably because limited dispersal ability seemed to make such a range implausible. Sponges from additional parts of the globe have been described as morphologically indistinguishable from *H. perlevis,* but in these cases taxonomists put

93   forth other names for these distant populations. For example, de Laubenfels described a
94   sponge he named *Hymeniacidon sinapium* from California in 1930 (de Laubenfels 1930,
95   1932). He acknowledged that "it is doubtful whether this is a new form", and went so far
96   as to suggest that species with the names "*sanguinea*, *luxurians*, *caruncula*, *heliophila*,
97   *sinapium*, and perhaps even more species" are in fact synonyms. Consistent with this
98   prediction, the European species *sanguinea* and *caruncula* have been synonymized with
99   *H. perlevis* (Van Soest et al. 2020a). The status of *H. luxurians* is unclear (Van Soest et al.
100   2020b), but the other two species, *H. sinapium* and *H. heliophila*, are still considered
101   valid. In the current work, I will present evidence that *H. sinapium* is conspecific with *H.*
102   *perlevis*, and that most sponges placed under the name *H. heliophila* are also *H. perlevis*.
103

104   When describing *H. sinapium* in California, de Laubenfels remarked on its impressive
105   ecological breadth. He described it as abundant in the "surf-beaten" intertidal throughout
106   Southern California, but also the most abundant sponge on the oyster beds in Newport
107   Bay (de Laubenfels 1932). He reported only one sample from subtidal depths, but his
108   subtidal sampling was limited, primarily via trawling. In contrast to this abundance in
109   Southern California, de Laubenfels was only able to locate a single specimen of this
110   species in Central California. This is notable because he was based at Hopkins Marine
111   Station in Monterey Bay (Central California) in the 1920s, and this was the area that he
112   studied most comprehensively at that time. A monographic report on Elkhorn Slough,
113   which drains into Monterey Bay, was published in 1935: it reports 4 species of sponges in
114   the estuary, but none similar to *H. sinapium* (MacGinitie 1935). This makes it unlikely
115   that this species was present in large numbers in Central California in the 1920s.
116   Subsequently, however, it has become a common species in intertidal portions of Elkhorn
117   Slough, which drains into Monterey Bay (Wasson et al. 2001), and it is also known from
118   Tomales Bay in Northern California (Wasson et al. 2001; Fuller and Hughey 2013).
119   Morphological (Sim 1985) and genetic (Hoshino et al. 2008) comparisons later confirmed
120   that a common *Hymeniacidon* in Korea, Japan, and China were the same species as those
121   in California, so it was proposed that *H. sinapium* was introduced to California from Asia
122   with oyster mariculture (Fuller and Hughey 2013). Though this is certainly possible, the
123   data I compile here illustrates that it may also be non-native in Asia. This species has
124   been said to occur in the Mexican Pacific (Hofknecht 1978) and the Galapagos Islands
125   (Desqueyroux-Faúndez and Van Soest 1997) as well, but genetic data are not yet
126   available from those populations.
127

128   The final species to consider, *H. heliophila* (Wilson 1911), is ascribed a substantial range
129   in the Western Atlantic, from the Gulf of Maine to Brazil (Muricy and Hajdu 2006;
130   Weigel and Erwin 2016; Van Soest et al. 2020c). Originally described as the most
131   abundant sponge in Beaufort Harbor North Carolina (Wilson 1911), it is also said to be
132   very common in the Caribbean (Diaz et al. 1993). A recent paper also found that an
133   indistinguishable sponge was the most common intertidal sponge present in the Bahía
134   San Antonio, Argentina, (Gastaldi et al. 2018). In this case, the authors opted to refer to
135   their samples by the name *H. perlevis*, as the Argentinian samples were indistinguishable
136   from ones in Northern Europe in genotype, habitat, and morphology (Gastaldi et al. 2018).
137

138 Here, I build on these results by 1) analyzing additional samples from Southern
139 California, which contains the type locality for *H. sinapium*, and 2) compiling all publicly
140 available genetic data (from 20 publications and several unpublished datasets). When
141 presented together, the data provide a compelling case for a single species ranging across
142 both the Atlantic and Pacific basins and the Northern and Southern hemispheres. Given
143 the limited dispersal capabilities of the species (Xue et al. 2009), the limited genetic
144 variation over most of its range (see below), and the historically documented range
145 expansion in California, these data are most consistent with an invasive spread via human
146 facilitation.
147
148 **Methods**
149
150 *Collections*
151 Sponges were located while SCUBA diving. Effort was made to photo-document all
152 sponge morphotypes present at each dive site, so that data on presence vs. absence could
153 be compiled. It should be noted, however, that search time was higher at some sites than
154 others, as shown in supplementary table 1. The search times listed are the total dive time,
155 cumulative across all dives at a site. This only approximates search effort, as some dives
156 were spent mainly searching and photographing sponges, while on others considerable
157 time was spent collecting samples. Collections were made by hand with a small knife.
158 Samples were placed individually in plastic bags while underwater, accompanied with
159 copious seawater. These bags were put on ice until preservation, which was generally
160 within 2-5 hours, but sometimes up to 12 hours. Samples were moved directly from
161 seawater to 95% ethanol; in most cases, the preservative was changed with fresh 95%
162 ethanol after 1-3 days, and sometimes changed again if it remained cloudy. Most samples
163 were photographed underwater with an Olympus TG5 before collection and
164 photographed again in the lab. These photos (and the microscope images discussed
165 below) accompany this paper as supplementary data and are also posted as georeferenced
166 records on the site iNaturalist.org. Two samples were collected during the "LA Urban
167 Ocean Bioblitz", and are present as vouchers at the Natural History Museum of Los
168 Angeles. Three other samples were deposited with the California Academy of Sciences in
169 San Francisco. Voucher numbers are shown in supplementary table 1. This table lists all
170 samples known or suspected to be *Hymeniacidon sinapium*. Note that the standard of
171 evidence is variable in each case, as indicated in the table (e.g. some were photographed
172 but not collected, and the ID is therefore tentative; see results for further details).
173
174 *Spicules*
175 Sponge spicules were examined by digesting soft tissues in bleach. A subsample of the
176 sponge was chosen, taking care to include both the ectosome and choanosome. This was
177 placed in a 1.5 ml microcentrifuge tube with household bleach for several hours, until
178 tissue appeared to be dissolved. With the spicules settled at the bottom of the tube, the
179 bleach was then pipetted off and replaced with distilled water; this was repeated several
180 times (I found that 2-3 water changes were sufficient for visualizing spicules with a light
181 microscope, but removing all the salt from the sample for other downstream applications
182 required 5 or more rinses and worked best when the final ones were done with absolute

4

183   ethanol). In some cases, samples were centrifuged at low speed to reduce settling time
184   between rinses, though this increased the proportion of broken spicules.
185
186   Spicules were imaged using a compound triocular scope and pictures were taken using a
187   D3500 SLR camera (Nikon) with a NDPL-1 microscope adaptor (Amscope). Pictures of
188   a calibration slide were used to determine the number of pixels per mm, and 20-30
189   spicules were then measured using ImageJ (Schneider et al. 2012). Spicules length was
190   determined in a straight line from tip to tip, even when spicules were curved or bent.
191   Spicules were selected randomly, so as to get an unbiased estimate of size distributions.
192   All raw data are available as supplementary table 2. I also imaged the spicular
193   architecture in cleared tissue sections. I used a razor blade to cut perpendicular sections
194   that were as thin as possible by hand, and removed the surface layer (ectosome) by hand
195   for a surface view. These sections, already in 95% ethanol, were soaked in 100% ethanol
196   for a short time and then cleared for several hours in Histoclear (National Diagnostics).
197
198   *Genotyping*
199   DNA was extracted from small subsamples, taking care to minimize contamination by
200   the many sponge-associated organisms that are often present. Some samples were
201   extracted with the Qiagen Blood & Tissue kit while the others were extracted with the
202   Qiagen Powersoil kit. The "barcoding" region of the cox1 gene was amplified using the
203   Folmer primers LCO1490 (GGTCAACAAATCATAAAGAYATYGG) and HCO2198
204   (TAAACTTCAGGGTGACCAAARAAYCA) (Folmer et al. 1994). A single attempt was
205   made to amplify a longer region using the primers from Rot et al. (Rot et al. 2006):
206   LCO1490 and COX1-R1 (TGTTGRGGGAAAAARGTTAAATT) but without success. A
207   portion of the 18S locus was amplified using the primers SP18aF
208   (CCTGCCAGTAGTCATATGCTT) and 600R18S (CGAGCTTTTTAACTGCAA)
209   (Redmond et al. 2013); the C2-D2 region of 28S was amplified using primers C2
210   (GAAAAGAACTTTGRARAGAGAGT) and D2 (TCCGTGTTTCAAGACGGG)
211   (Chombard et al. 1998). All primer sequences are listed 5' to 3'. PCR was performed in a
212   Biorad T100 thermocycler with the following conditions: 95C for 3 min, followed by 35
213   cycles of 94C for 30 sec, 52C for 30 sec, 72C for 1 min, followed by 72C for 5 minutes.
214   The C2-D2 28S region was amplified with a 57C annealing temperature instead of 52C.
215   PCR was performed in 50 ul reactions using the following recipe: 24 μL nuclease-free
216   water, 10 μL 5x PCR buffer (Gotaq flexi, Promega), 8 μL 25mM MgCl, 1 μL 10mM
217   dNTPs (Promega), 2.5 μL of each primer at 10 μM, 0.75 μL bovine serum albumin (10
218   mg/ml), 0.25 μL Taq (Gotaq flexi, Promega), 1 μL template. ExoSAP-IT (Applied
219   Biosciences) was used to clean PCRs, which were then sequenced by Functional
220   Biosciences (Madison, Wisconsin) using Big Dye V3.1 on ABI 3730xl instruments. All
221   PCR products were sequenced in both directions, and a consensus sequence was
222   constructed using Codon Code v.9 (CodonCode Corporation). Blastn was used to verify
223   that the resulting traces were of sponge origin; all sequences have been deposited in
224   Genbank as accessions MT007958-MT007960 (cox1), MT001298 (18S), and MT006362
225   and MT422190 (28S). See supplementary table 3 for additional details and information.
226
227   *Genetic analysis*

228   I retrieved all sequences with high sequence similarity to *H. perlevis* from Genbank. I
229   used the NCBI taxonomy browser to compile all data from samples identified as *H.*
230   *perlevis, H. sinapium, H. heliophila*, and *H. flavia.* Together, these data are from 20
231   different publications and several datasets that were deposited in Genbank but never
232   published (Erpenbeck et al. 2002, 2004, 2005, 2006, 2007; Park et al. 2007; Hoshino et al.
233   2008; Turque et al. 2008; Erwin et al. 2011; Alex et al. 2012, 2013; Morrow et al. 2013;
234   Redmond et al. 2013; Thacker et al. 2013; Fuller and Hughey 2013; Jun et al. 2015;
235   Miralles et al. 2016; Weigel and Erwin 2016; Gastaldi et al. 2018; Regueiras et al. 2019).
236   I also retrieved all samples identified as *Hymeniacidon* sp. and checked these and other
237   sequences using blastn for similarity to *H. perlevis/sinapium/heliophila*. Only four of
238   these (JN093018 and KU697715-KU697717), all identified as *Hymeniacidon* sp., were
239   closely related to the other samples, and all appeared to be identical to other sequences
240   within the *H. perlevis* clade. These four unidentified samples were not included in
241   downstream analyses.
242
243   I was not able to use all sequences in every analysis because of differences in the
244   sequenced portion of the gene or a lack of information regarding collecting location.
245   Importantly, no samples were excluded simply because they showed discordant patterns
246   of sequence variation. Supplementary table 3 lists every Genbank accession found,
247   indicates which were included in each analysis, and explains the reasons why any were
248   excluded. Some reads were included in the phylogenetic analysis, which could tolerate
249   unequal read lengths, but not the haplotype network, which included only samples with
250   complete data over the entire alignment. Sequence alignments were produced in Codon
251   Code v.9 (CodonCode Corporation). Haplotype networks were produced using the
252   minimum spanning method (Bandelt et al. 1999) as implemented in Popart (Leigh and
253   Bryant 2015). Phylogenies were estimated with maximum likelihood using IQ-Tree
254   (Nguyen et al. 2015; Trifinopoulos et al. 2016). I used a GTR model of sequence
255   evolution and used the Ultrafast bootstrap (Hoang et al. 2018) to measure node
256   confidence. Phylogenies were produced from the IQ-Tree files using the Interactive Tree
257   of Life webserver (Letunic and Bork 2019). Figures were made ready for publication
258   using R (r-project.org) and/or Gimp (gimp.org).
259
260   **Results**
261
262   *Status in Southern California*
263   Little data has been published about the distribution of *Hymeniacidon sinapium* in
264   California outside of bays and estuaries. Past surveys have focused on intertidal habitat
265   and/or subtidal sampling via deep water trawl (de Laubenfels 1932; Sim and Bakus 1986;
266   Bakus and Green 1987; Green and Bakus 1994). It was therefore unknown if *H. sinapium*
267   is present in kelp forest ecosystems. I searched for it using SCUBA at 47 sites in
268   Southern and Central California (table S1). Subtidal sites were shallow rocky reefs except
269   for two locations which were oil platforms. Subtidal sites include four marine protected
270   areas along the mainland coast and three marine protected areas within the Channel
271   Islands National Marine Sanctuary. Six of the sites are also field sites within the Santa
272   Barbara Coastal Long-Term Ecological Research Network (sbclter.msi.ucsb.edu).

273   Though the survey was focused on kelp forest habitats, I also checked two intertidal sites
274   and floating docks in two harbors, as shown in table S1.
275
276   The distribution of *H. sinapium* in the Channel Islands region, where sampling was most
277   comprehensive, is shown in figure 1. I found the sponge at 8 of 19 mainland reefs in
278   Southern California, including both mainland marine protected areas investigated in
279   Southern California (Naples and Campus Point Marine Protected Areas). In only one
280   location (Carpinteria Reef) did I find *H. sinapium* growing on rock; in all other locations
281   it was largely buried in sediment, with projections extending into the water column. In
282   contrast to its prevalence on the Southern California mainland, I did not find it at any
283   island sites. This difference seems unlikely to be due to dispersal limitation because
284   island and mainland sites have high connectivity (Watson et al. 2010). It is more likely
285   due to the ecological differences between sites: none of the island sites investigated had
286   areas with the fine silty sediment where the sponge was most common on the mainland.
287   Though silty sites at the islands may simply have been unsampled in this survey, it is
288   likely they are less common than on the mainland. For example, satellite data show that
289   particles at the islands are less prone to resuspension by wave action (Freitas et al. 2017).
290   An intertidal survey of island sites in the 1970s did find *H. sinapium* at both San Miguel
291   and Santa Rosa Islands (Bakus and Green 1987). It has also been reported from the more
292   Southern islands of Catalina and San Clemente, which were barely sampled by my survey
293   (Sim and Bakus 1986; Bakus and Green 1987). To the North, in Central California, I only
294   surveyed three subtidal sites. I did not find *H. sinapium* in any of these Central California
295   sites, nor did I find it at the few intertidal sites, floating docks, or oil rigs that were
296   checked.
297
298   Together, my recent collections and the published intertidal and bay surveys in California
299   produce a portrait of a species that thrives in a wide variety of conditions, from bays to
300   the rocky intertidal to the kelp forest (Lee et al. 2007). It seems most abundant in the
301   intertidal in some bay habitats with a muddy substrate and high sedimentation, and seems
302   more common in the kelp forest where fine sediment is found. These data are completely
303   consistent with the published descriptions of habitat preferences for *H. perlevis* in Europe
304   (Erpenbeck and Van Soest 2002) and *H. heliophila* in the Western Atlantic (Weigel and
305   Erwin 2016).
306
307   *Gross Morphology of kelp forest samples*
308   All but one of the newly collected samples were found embedded in sediment with
309   irregular projections extending into the water column. These projections varied from
310   stout cone-shaped or bag-shaped oscula to long, tendril-like digitations. One sponge was
311   found unburied, growing on rock. It lacked projections and instead resembled
312   *Halichondria panicea* (its identity was confirmed with spicule and DNA data, presented
313   below). All samples had a fleshy consistency, with the rock-dwelling sponge somewhat
314   firmer. Color varied from yellow to yellowish-orange in the field. Field photos are
315   available for 8 samples in the supplementary data accompanying this paper, and are also
316   available at iNaturalist.org.
317

7

318   I was interested in whether these sponges could be identified in the field and therefore
319   monitored using roving diver surveys or photo transects. These samples were collected as
320   part of an ongoing project to characterize the diversity of kelp forest sponges, with over
321   500 samples collected to date. This is one of the first surveys of sponges in California via
322   SCUBA, and the first with extensive field photos of specimens that have also been
323   analyzed morphologically. Though the bulk of these data will be published elsewhere,
324   comparisons to date indicate that *H. sinapium* is the only sponge in these habitats that
325   grows by extending irregularly shaped projections out of silty sediment. Though this
326   morphology is certainly known from other species, *H. sinapium* was the only sponge with
327   this morphology found within the sampling effort shown in supplementary table 1. This
328   indicates that this morphology, when found in the Southern California kelp forest, is
329   strongly suggestive of the presence of this species. The most similar species found to date
330   is *Polymastia pachymastia*: as the name suggests, this sponge is covered in nipple-like
331   projections. This sponge was also found covered in sediment, with only the projections
332   visible. However, these projections tend to be uniform in shape and regularly spaced in *P.*
333   *pachymastia*, which contrasts with the irregularly spaced and morphologically various
334   projections seen in *H. sinapium.* The projections are also nearly white in *P. pachymastia*,
335   while they vary from yellow to nearly orange in *H. sinapium.* The rock-dwelling *H.*
336   *sinapium* found at Carpenteria Reef, however, would be more challenging to identify
337   from field photos, as it is very similar to other Halichondridae found in the survey.
338
339   *Spicular morphology*
340   I characterized the spicules of 9 samples to confirm their identity and compare them to
341   published data. All spicules were styles: tapered to a point at one end, and rounded at the
342   other. Width was usually uniform over the entire length, but a small minority had faint
343   swelling at or near the rounded end. This was manifest as a very weak swollen head
344   including the end (similar to the head of a match), or more commonly as a swollen band
345   near the head end (like a bead on a string). Most were somewhat curved or bent. The
346   skeleton of one sample was investigated further using hand-cut sections cleared with
347   Histoclear. Spicules in perpendicular sections through the choanosome formed wavy,
348   meandering tracts, the largest of which were about 30 μm wide. Spicules were also found
349   outside the tracts pointing in all directions (referred to as a "confused" arrangement in
350   sponge taxonomy). Surface sections revealed that the ectosome of the sponge was filled
351   with spicules that appeared to be tangential (parallel to the sponge surface) and also
352   "paratangential" (at an angle to the surface of less than 90 degrees). These spicules were
353   in messy bundles that formed an approximate mesh on the surface of the sponge. Table 1
354   shows measurements of spicules as compared to values published in other studies of
355   *Hymeniacidon*. Newly collected data are consistent with published data from *H. sinapium,*
356   *H. perlevis,* and *H. heliophila,* as well as *H. fernandezi* (Thiele 1905) from Chile (for
357   which no genetic data is yet available). The arrangement of spicules in cleared sections is
358   also congruent with the spicular architecture described for *H. perlevis* and other species
359   (Erpenbeck and Van Soest 2002). Photos of tissue sections and spicules are available as
360   supplementary data.
361
362   *Genetic analysis*

8

363   I sequenced the newly collected samples at the cox1 locus (3 samples), the 18S rDNA (1
364   sample) and the 28S rDNA (2 samples). A sample of the *Hymeniacidon sinapium*
365   holotype was also loaned to me by the Smithsonian Natural History Museum: despite
366   repeated attempts, I was unable to amplify DNA from this sample. This was not
367   surprising, as it was collected in 1926 and little is known about its initial preservation.
368

369   I mined Genbank for all DNA data available for *H. perlevis, H. sinapium,* and *H.*
370   *heliophila* (see methods). I generated sequence alignments for four loci: cox1, 18S rDNA,
371   28S rDNA, and a locus spanning the first intergenic transcribed spacer, the 5.8S rRNA,
372   and the second intergenic transcribed spacer (hereafter referred to as the ITS). No other
373   locus had more than 2 sequences available in Genbank from any of these taxa.
374   Preliminary phylogenies indicated that sequences of *Hymeniacidon flavia* were more
375   closely related to the clade containing my target species than anything else in Genbank.
376   When available, these sequences were included for comparison.
377

378   Figure 2 shows the haplotype networks for the three loci with the most data. A large
379   dataset was available for 226 base pairs at the ITS locus. A set of 271 sponges from Japan
380   and Korea contained little genetic variation, as previously described (Park et al. 2007;
381   Hoshino et al. 2008). Samples from Northern, Central, and Southern California were all
382   identical to the most common Asian haplotype, as were 9 of 10 samples of *H. heliophila*
383   from the Eastern United States. These include samples from Alabama, Florida, and North
384   Carolina. This last sequence read is the only one available that is identified as coming
385   from this state, which contains the type location for *H. heliophila*. As a useful
386   comparison to the diversity in 298 samples of *perlevis/heliophila/sinapium*, a population
387   sample of 212 *H. flavia* are shown. These are all from Japan, yet they contain a similar
388   amount of diversity as the worldwide sample from *H. perlevis/heliophila/sinapium*. In
389   contrast to the lack of divergence between *H. perlevis, H. heliophila,* and *H. sinapium,*
390   the *H. perlevis/heliophila/sinapium* clade is well demarcated compared to other species.
391   Sequences from closely related *H. flavia* differ by 8.4—9.7%. Attempts to align other
392   published Halichondridae sequences at this locus failed due to very high sequence
393   divergence.
394

395   A large mitochondrial dataset is also available at the Folmer barcoding region of the cox1
396   locus (571 bp; fig. 2). A single haplotype was shared among populations from China,
397   Korea, Southern California, Florida, and Portugal. Samples from Argentina contained
398   only 1–2 differences (99.6% identity) compared to this world-wide haplotype. The only
399   sample that is more than 0.5% divergent from this common haplotype, out of all *H.*
400   *perlevis/sinapium/heliophila* available, is a single sequence from Rio de Janeiro, Brazil
401   (1.2% divergent; top of fig. 2). No morphological description of this sample is available
402   in the related publication (Turque et al. 2008), but it states that vouchers were deposited
403   in the Museu Nacional, Universidade Federal do Rio de Janeiro. Personal
404   communications with Guilherme Muricy at the Museu Nacional indicate that this sample
405   matches *H. heliophila* in both gross morphology and spicular morphology, as described
406   in Muricy and Hajdu 2006 (p. 53). This sample is discussed further below.
407

408   Though I found that *H. perlevis, H. heliophila, and H. sinapium* shared an identical
409   genotype at 571 bp of cox1, the cox1 locus is known to have a slower evolutionary rate in
410   sponges than most other animals (Huang et al. 2008). Additional interspecific sequence
411   comparisons provide context for this lack of divergence. First, the *H.*
412   *perlevis/heliophila/sinapium* clade is differentiated from its closest relative, *H. flavia*,
413   with 2.5 - 4.5% sequence divergence (2.5 - 3.7% if the sample from Rio de Janeiro is
414   excluded). Additionally, Genbank data illustrate that most named species in the
415   Suberitida are genetically distinguishable at cox1. Excluding the *H.*
416   *perlevis/heliophila/sinapium* clade, a cox1 sequence is available from 58 vouchers across
417   39 species for the order Suberitida. I determined sequence divergence between each
418   voucher and the most similar sequence from a different species. In only one case are two
419   sequences identical over a continuous 500 bp or more. This case is for sequences
420   identified as *Suberites pagurorum* and *Suberites domuncula*, which are themselves
421   members of a species complex in need of taxonomic revision (Solé-Cava and Thorpe
422   1986). Across all 58 comparisons, average sequence divergence to the most similar
423   conspecific voucher was 3.7% (standard deviation = 3.2%).
424

425   Less data is available for the 18S and 28S rDNA loci, but the 18S locus once again
426   illustrates the genetic similarity of Atlantic *H. perlevis/heliophila* populations and Pacific
427   *H. sinapium* populations (figure 2). Over the aligned 1,642 bp, samples from China
428   shared an identical haplotype with samples from Argentina. A sample of *H. perlevis* from
429   Ireland differs from this common haplotype by only a single base pair (this is the closest
430   available data to the type locality for *H. perlevis*, which is the Devon Coast in England).
431   Only a single data point has any notable divergence: a sponge identified as *H. heliophila*
432   from the USA. This sample is separated from all others by 12 substitutions (0.7%
433   divergence). I created a phylogeny including selected Halichondridae to place this
434   divergence in context (figure 3). While all other sequences of *H.*
435   *perlevis/heliophila/sinapium* form a clade, this USA sample is as divergent as other
436   distinct species. The interior nodes of this phylogeny are not well resolved, but it is clear
437   that this sequence is an outlier and likely from a different species. This sample (NCI217,
438   Smithsonian voucher #0M9G1074-H) is part of a collection of sponges for the National
439   Cancer Institute deposited at the Smithsonian Museum of Natural History (Redmond et al.
440   2013). It was collected by the Coral Reef Research Foundation in the Florida Keys (Key
441   Largo, from mud substrate), and identified by Belinda Glasby (William Moser,
442   Smithsonian Museum of Natural History, pers. comm.). It is discussed further below.
443   Divergence between all other sequences in the *H. perlevis/heliophila/sinapium* clade and
444   the most closely related species available (*Halichondria bowerbanki*) is 1.4 - 1.8%. This
445   is less divergence than at the ITS and cox1 loci, indicating lower power to discriminate
446   among species on a per-base basis. The aligned region is 3 times longer than cox1 and 7
447   times longer than ITS, however.
448

449   The D3–D5 region of 28S also allowed for an interesting comparison (figure 3). The only
450   data available at this locus is from two European samples and two from the Florida Keys,
451   USA. One of the Florida sequences is from the same isolate as the outlier at 18S
452   (NCI217), while the other sample is from the same collection (NCI083, Smithsonian
453   voucher #0M9G1369-A) (Thacker et al. 2013). It was collected in the Florida Keys

454 (Marquesas Key, sand substrate; William Moser, pers. comm.) In agreement with the 18S
455 data, these samples do not appear to be from the same species as the European ones.
456
457 **Discussion**
458 Genetic data provide strong support for the synonymy of *H. perlevis* and *H. sinapium*.
459 The type locality for *H. sinapium* is Newport Bay, in Southern California (de Laubenfels
460 1932). Previously, the only genetic data from Southern California was from a single
461 sample from Mission Bay, roughly 140 km to the South of the type location. To this I
462 have added additional data from Santa Barbara County (200 km North of the type
463 location). All of these samples are genetically identical to samples of *H. perlevis*. Indeed,
464 there is no appreciable genetic divergence between any sample from California, Japan,
465 Korea or Europe. One of the loci investigated, the ITS locus, is the fastest-evolving locus
466 regularly used in sponge systematics. It evolves too fast to be informative above the level
467 of family (Wörheide et al. 2004), and is more commonly used to infer population
468 structure within species (Wörheide et al. 2002, Duran et al. 2004). The cox1 locus
469 evolves more slowly, but I found that it still shows an average of 3.7% sequence
470 divergence within this order of sponges, and a published data spanning phylum Porifera
471 found an average of 4.9% divergence (Huang et al. 2008). The 18S locus evolves more
472 slowly still, but the longer alignment at this locus was sufficient to differentiate other
473 species in the family, as shown in the phylogeny. It remains possible that genomic
474 analyses could reveal reproductively isolated groups that are not differentiated at these
475 particular genes (e.g. Turner et al. 2008). However, there is no reason to assume any such
476 "cryptic species" would be associated with the geographic regions previously ascribed to
477 *H. perlevis* and *H. sinapium*. I therefore formally propose that *H. sinapium* de Laubenfels
478 (1930) be considered a junior synonym of *H. perlevis* Montagu (1814).
479
480 It is possible that *H. heliophila* is also a junior synonym of *H. perlevis,* but some
481 ambiguity remains. Genetic data illustrate that the majority of samples identified as *H.
482 heliophila* are in fact *H. perlevis*, including the only one from North Carolina, which
483 contains the type location. The two National Cancer Institute vouchers from Florida,
484 however, appear to be from a different species. One cox1 sequence from Brazil is also
485 modestly divergent, and could be from another species. It is possible that there are two
486 morphologically similar *Hymeniacidon* within the range ascribed to *H. heliophila*,
487 mirroring the case of *H. sinapium* and *H. flavia* in Japan and Korea. Though further work
488 will be required to determine if the name *H. heliophila* is valid, is it clear that the most
489 common sponge matching its description is in fact *H. perlevis*, whose range therefore
490 includes North Carolina, Florida, Alabama and Argentina at the very least.
491
492 Ecological and morphological data also support these far-flung populations being within
493 the same species. It should be noted, however, that there is another species that is
494 morphologically similar yet genetically distinct. The genetic outgroup to the *H.
495 perlevis/heliophila/sinapium* clade is *H. flavia*, known from Japan and Korea (Park et al.
496 2007; Hoshino et al. 2008). This species is sympatric with *H. sinapium* in Japan and
497 Korea, and cannot be distinguished from it based on spicular morphology. These species
498 can only be identified using genetic data, color when alive, or larval morphology (Sim
499 and Lee 2003; Hoshino et al. 2008). This illustrates that it may be difficult to resolve the

11

500   taxonomy of *Hymeniacidon* without genetic data. As pointed out by Gastaldi et al.
501   (Gastaldi et al. 2018), there are additional species with morphological descriptions
502   matching *H. perlevis*, but the existence of *H. flavia* shows that confidently determining
503   which are synonyms will require DNA data.
504
505   Data on larval biology are available for *H. perlevis*, and they support a hypothesis of
506   recent range expansion via human facilitation. Larvae of *H. perlevis* are non-tufted
507   parenchymella, and do not appear to differ in their dispersal time compared to other
508   studied species (Xue et al. 2009). In the lab, all larvae stopped swimming and were
509   exploring the benthos by 19 hours after release, and all had settled by 43 hours. In
510   unfavorable conditions the larvae may travel farther: under high artificial illumination
511   (which increased mortality), sponge larvae swam for a maximum of 24 hours and some
512   were still exploring the benthos when the experiment was terminated at 68 hours. These
513   data are consistent with the larval ecology of other sponges (Maldonado 2006). It
514   therefore seems unlikely that the larvae of this species have exceptionally higher
515   dispersal than to other sponges.
516
517   The data do not seem sufficient to form a strong hypothesis about the native range of this
518   species. It seems unlikely to be California, as almost no genetic variation was found at
519   the ITS or cox1 loci in that region. Moreover, the species has likely undergone range
520   expansion in California between the 1920s and the present. Europe is perhaps the most
521   likely source, as we know it was present there in the early 1800s. The genetic diversity at
522   cox1 in Portugal seems notably higher than the diversity at the ITS locus in Asia, though
523   better support would clearly come from comparing data from the same locus.
524
525   Future work will be needed to understand what impact this species has on host
526   ecosystems. Its abundance in some habitats seems to make impacts likely, if for no other
527   reason than the occupation of space (Wasson et al. 2001). It is also notable that it has
528   successfully colonized the kelp forests in California, which have been relatively resistant
529   to invasion (Steneck et al. 2002). Within these kelp forests, my observations suggest that
530   this species can be monitored, if imperfectly, using roving diver surveys or photo
531   transects. Though some sponges would require follow-up confirmation in the lab, this
532   might allow the existing large-scale monitoring efforts in California to include this
533   species (Claisse et al. 2018; Miller et al. 2018).
534
535   The work presented here builds on the excellent previous work of many authors, and
536   aspects of the pattern I describe have certainly been recognized by others. Hoshino et al.
537   (2008) and Gastaldi et al. (2018) both remarked upon the similarity of *Hymeniacidon* in
538   the *H. perlevis* clade, though in both cases they referred to this clade as a species complex
539   that might be synonymized in the future. Others have simply started referring to their
540   samples by the senior synonym *H. perlevis*, even if they are within the range ascribed to
541   one of the other taxa (Xue et al. 2009; Gastaldi et al. 2018). I build on these earlier efforts
542   by adding data from Southern California and, for the first time, presenting all the genetic
543   data from other projects in one analysis. I recommend synonymizing *H. sinapium* and *H.*
544   *perlevis*, and recognizing that *H. perlevis* is an invasive species with a global distribution.

545 My hope is that recognition of the unusual distribution and abundance of this species
546 motivates further work into its ecology and ecological impacts.
547

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Table 1. Morphological data from newly collected samples with comparison data from the literature.

Sample	Date	Collection location	N	Style length	Style width
TLT550	1/7/20	Arroyo Quemado	25	151 - 262 - 336	3 - 5 - 7
TLT79	7/1/19	Tajigus	26	121 - 223 - 322	3 - 5 - 7
TLT87	7/1/19	Tajigus	21	147 - 216 - 326	2 - 5 - 8
TLT109	7/1/19	Tajigus	32	138 - 289 - 415	4 - 6 - 8
TLT339	8/30/19	Coal Oil Point	25	160 - 283 - 376	2 - 5 - 6
TLT247	7/31/19	Carpinteria	20	129 - 223 - 319	4 - 5 - 7
TLT129	7/31/19	Carpinteria	36	116 - 246 - 334	3 - 5 - 7
TLT15955	8/22/19	Redondo Barge	25	127 - 235 - 407	3 - 5 - 7
TLT349	8/23/19	Resort Wall	23	163 - 264 - 351	2 - 5 - 12
H. sinapium[1]	-	Elkhorn Slough, CA	-	115 - 460	3 - 12
H. perlevis[2]	-	Wales	-	152 - 475	3 - 12
H. perlevis[3]	-	South Africa	-	155 - 337	7
H. perlevis[4]	-	New Zealand	-	189 - 329	2 - 10
H. heliophila[5]	-	Caribbean	-	130 - 450	3 - 10
H. fernandezi[6]	-	Chile	-	200 - 340	3 - 10

Date = collection date, N = number of spicules measured, spicule dimensions given in format min - mean - max; when only two numbers are shown they are min - max.
Sources: 1: Lee et al. 2007; 2: Erpenback & Van Soest 2002; 3: Samaai & Gibbons 2005; 4: Bergquist 1970; 6: Thiele 1905.

805 **Figure 1.** Collection locations in the Southern California Channel Islands region. Sites
806 where *H. sinapium* were found (black) and not found (white) are shown. The two sites
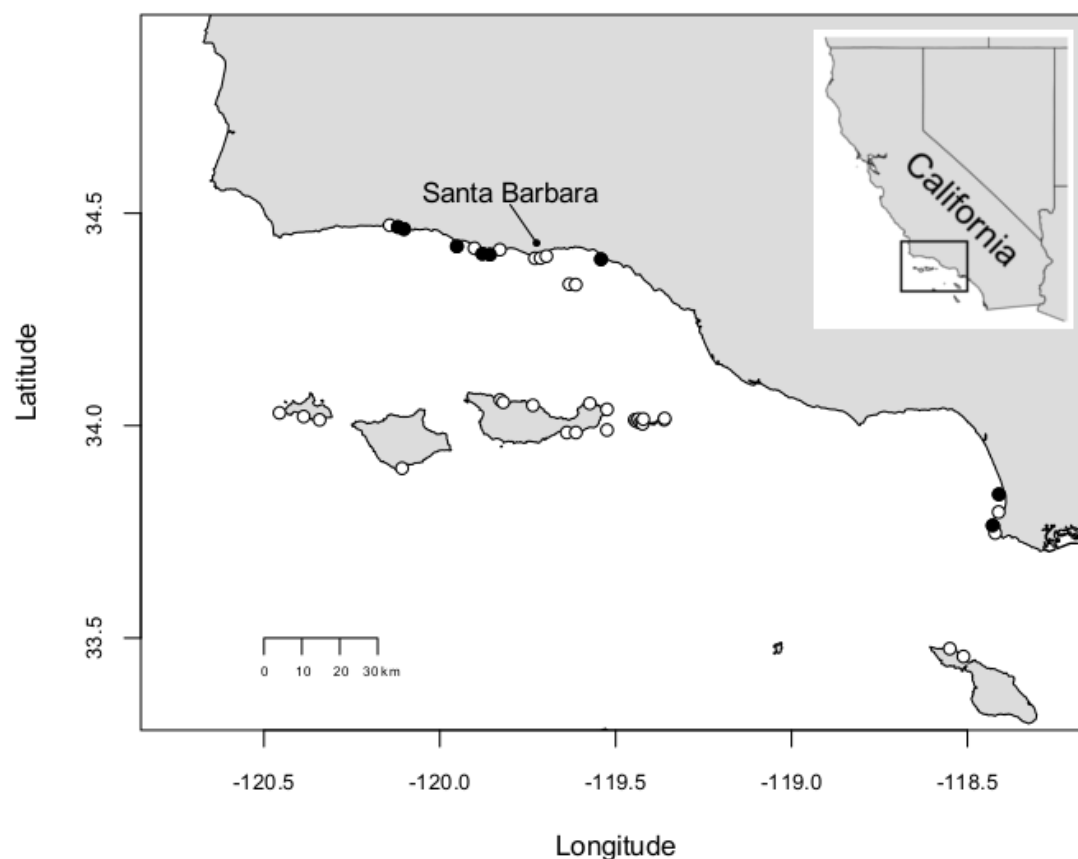807 away from the coastline are oil platforms. Collection sites in Central California are not
808 shown.
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814 **Figure 2.** Minimum-spanning genotype networks for three loci. Samples are coded by
815 collection location, regardless of whether they were identified as *H. perlevis, H. sinapium,*
816 or *H. heliophila*. Closely related *H. flavia* are shown for comparison where available; all
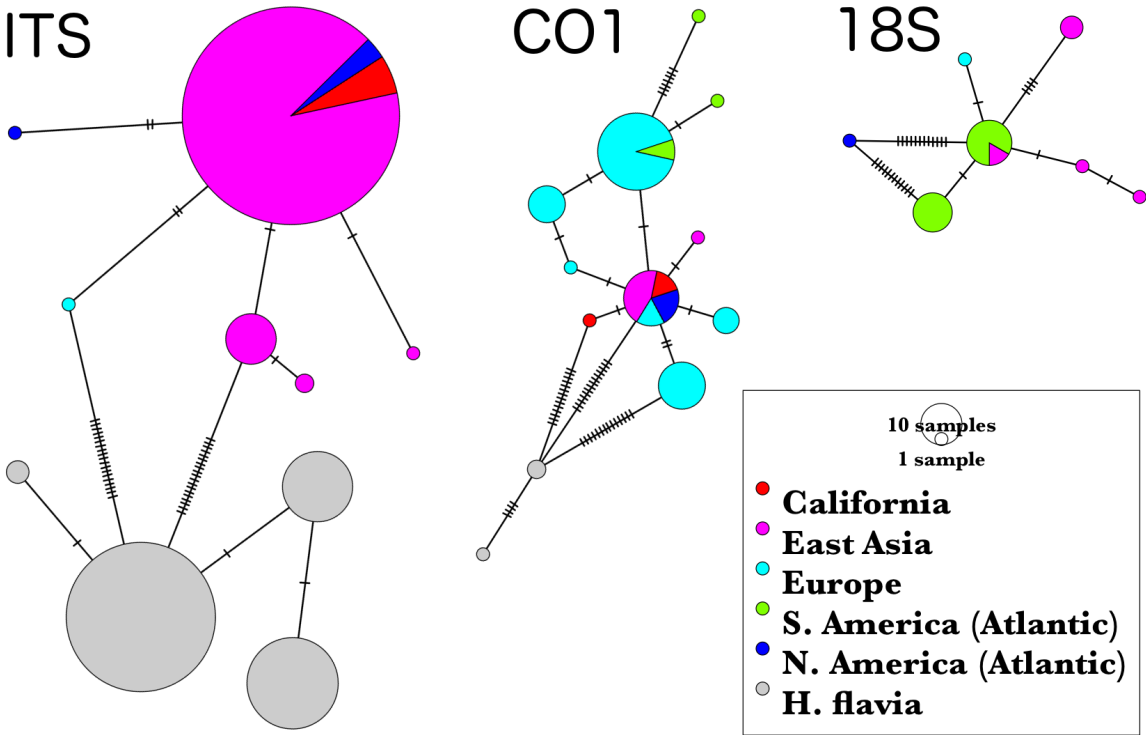817 data for this species is from Japan and Korea.
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824   **Figure 3.** Phylogenies for the 18S and 28S loci. All samples identified as *H. perlevis, H.*
825   *sinapium* and *H. heliophila* are shown in bold with localities. Selected other
826   Halichondridae are shown for comparison, with *Suberites domuncula* specified as the
827   outgroup. Genbank accession numbers are also shown. Ultrafast Bootstrap support is
828   shown for all nodes with > 50% support.
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