From Great Britain to the South African coast: the invasive sponge *Hymeniacidon perlevis* (Montagu, 1814) (#72071)

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From Great Britain to the South African coast: the invasive sponge *Hymeniacidon perlevis* (Montagu, 1814)

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Background. Intertidal rocky shore surveys along the South African coastline (~3 000 km) have demonstrated the presence and abundance of the encrusting orange sponge *Hymeniacidon perlevis* (Montagu, 1814), a well-known globally invasive species. Its species identity, however, is in doubt pending molecular confirmation. The purpose of this investigation was to confirm the presence of *H. perlevis* in South Africa, determine its distribution in the intertidal rocky shore ecosystem, and compare its genetic diversity to other congeners in South Africa and globally. **Methods.** We surveyed the entire South African coastline from west to east at 47 rocky shore sites, which confirmed that populations of this sponge occur in most wave and non-wave exposed mid-shore intertidal habitats between Lüderitz (Namibia) and Dwesa/Cwebe (South Africa) and that its current distribution spans over 2000 km of coastline. It does not occur on the intertidal rocky shores of the subtropical South African east coast. DNA sequences of the nuclear rDNA internal transcribed spacers (ITS1) and the COI mitochondrial gene were obtained from 61 samples, from populations separated by distances of 20 to 2500 km, spanning from the west to the south coasts of South Africa. **Results.** The sequences were compared to congeners in GenBank and were found to be 99-100% identical to the other *H. perlevis* and *H. sinapium* sequences. These data conclusively document the presence of *H. perlevis* in South Africa as well as assign the species to a relatively wide geographical range spanning more than 2 000 km. *This species* should thus be added to the invasive list.

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Abstract

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Keywords

Porifera, rocky intertidal area, introduced species, barcoding, population structure, haplotype networks, COI, ITS

Introduction

Biological invasions are becoming more common due to global environmental change and undetected transportation associated with increased global trade and other anthropogenic activities (Dijoux, Viard & Payri, 2014). Invasive species have widespread, disjunct distributions and are typically abundant in their non-native environments (Lloret et al., 2004). Marine alien invasive species are recognized as one of the major drivers of global biodiversity loss (Molnar et al., 2008; Keller et al., 2011).

Coastal marine ecosystems are particularly prone to species introductions especially intertidal rocky shores around the world. This ecosystem has been severely impacted by invasive species, particularly by mussels, crabs, bryozoans, ascidians, algae and barnacles (Mackie, Keough & Christidis, 2006; Haydar et al., 2011; Pérez-Portela et al., 2013; Lee et al., 2014; Pfaff et al., 2022). A well-known example is the world-wide dispersal of the brown mussel *Perna perna* (Linnaeus, 1758) from its indigenous range in the Red Sea, eastern and southwestern Africa to the Gulf of Mexico, the Atlantic coast of South America, southern India, and Sri Lanka (Douek et al., 2021). The populations in the western Mediterranean and the adjacent Atlantic coast are considered cryptogenic (Douek et al., 2021). Another example is *Mytilus galloprovincialis* (Lamarck, 1819), indigenous to the Mediterranean, Adriatic, and Black Seas which has since expanded to Japan, South Africa, China, the United States, Canada, Korea, Australia, and Mexico (Zardi et al., 2007). Furthermore, poleward range shifts, whereby tropical species move into subtropical and temperate regions, have become increasing common (VanDerWal et al., 2013; Lourenço et al., 2017), facilitating a new suite of invasions via climate-mediated thermal shifts (Haydar et al., 2011; Carballo et al., 2013; Carbonell, Wang & Stoks, 2021).

Hymeniacidon perlevis (Montagu, 1814) are the most widely distributed sponges in the intertidal (up to 3 m above the low tide line) (Gastaldi et al., 2018; Regueiras et al., 2019; Turner, 2020, de Voogd et al., 2022) and this range is partly due to several introduction events (Turner, 2020). This very prolific encrusting orange sponge was first described from Devon, southwest England, Northern Hemisphere as *Spongia perlevis* Montagu, 1814. The type locality has very similar environmental conditions to the west coast of South Africa (Smit et al., 2013). This is one of the most common intertidal species of Europe (Erpenbeck and Van Soest, 2002).

The original description is very confusing and vague, with no figures of the diagnostic characters (see Montague, 1814, pp. 86). Erpenbeck & Van Soest (2002) described *H. perlevis* as thickly encrusting and orange-brown in the dry condition. The surface is covered with irregularly shaped short papillae and small digitations. Consistency is fleshy, with the colour in life varying from orange to blood-red depending on geographical location (Ackers, Moss & Picton, 2007). The ectosomal skeleton is a dense crust, being paratangential as opposed to having a strictly tangential arrangement. The choanosomal skeleton is less dense, with wispy bundles following a wavy course to the surface, and many single spicules in confusion. Styles, slightly curved, often with a faint tylote swelling subterminally, 152–475 × 3–12 μm.

To date, genetic and morphological data support a distribution that includes the Atlantic coast of Europe (Portugal, Spain) to the Mediterranean (Adriatic Sea) and Canary Islands (Ackers, 2007; Alex et al., 2012), the Atlantic coasts of North and South America (Florida, Argentina), the Pacific coast of North America (California) and Asia (Japan, China and Korea) (Erpenbeck and Van Soest, 2002; Gastaldi et al., 2018; Schwindt et al., 2020; Turner, 2020) (Fig. 1). The species was also described from New Zealand, Australia, West Africa, Namibia, South Africa, Canada (British Columbia) and the Pacific coast of South America (Bergquist, 1970; Erpenbeck and Van Soest, 2002; Samaai and Gibbons, 2005; Regueiras et al., 2019; Harbo et al., 2021) (Fig. 1), but no genetic data are available for these populations. Apart from this, *H. perlevis* also has 27 synonymized names from approximately 100 localities globally (de Voogd et al., 2022). It is thought to have been introduced to the Mediterranean, Adriatic, and Black Seas, presumably from Southern England, but also occurs in Japan, China, the United States, Argentina, Korea; the vector is unknown (Turner, 2020). However, its widespread presence today in California, Argentina, Korea, and China may well be explained by introductions from other areas (Turner, 2020). In South Africa, *H. perlevis*, is not listed as an invasive species (Robinson et al., 2005; Mead et al., 2011).

To date, no studies have been conducted in South Africa to determine the taxonomic status of *H. perlevis*, commonly known as “the crumb of bread sponge”. This species is conspicuous to the South African intertidal shores and was first recoded from Saldanha Bay, False bay and Hout Bay during the Scotia’s expedition in the early 1900 by Stephens (1915). Stephens (1915) identified and described two Saldanha Bay species respectively that are now within the genus *Hymeniacidon*: *Halichondria caruncula* (Bowerbank, 1858), which is regarded

as a junior synonym of *Hymeniacidon perlevis* (Montagu, 1814) (Ackers, Moss & Picton, 2007; de Voogd et a., 2022), and *Leucophloeus styliferus* Stephens, 1915, which is now accepted as *Hymeniacidon stylifera* (Stephens, 1915) (see de Voogd et al., 2022). Penrith and Kensley (1970) and Day (1974) reported the presence of *H. perlevis* in South Africa based on the published works by Stephenson (1938, 1944, 1948). Additionally, Branch et al. (2005) listed *H. perlevis* in their field guide to the region and recorded its distribution as occurring from Port Nolloth on the west coast to East London on the east coast. *Hymeniacidon perlevis* was first described in detail from the west coast of South Africa by Samaai & Gibbons (2005). During the 2001 Saldanha Bay port survey (Awad et al., unpublished Report), the species was not recoded, no Porifera were included in in the geographical analyses of Emanuel (1992) and Awad et al. (2002). Penrith and Kensley (1970a) recorded the presence of *H. perlevis* from the intertidal region of Lüderitz, Namibia. From Rocky Point to the Kunene River north of Lüderitz, the species was not recorded (Penrith and Kensley, 1970b; Kensley & Penrith, 1980).

Hymeniacidon perlevis larvae are lecithotrophic, ciliated to some extent, with a relatively short planktonic life resulting in low dispersal capacity (Maldonado, 2006; Xue et al., 2009). The regional or global distribution has been attributed to maritime traffic (Gastaldi et al., 2018; Turner, 2020). The difficulty in distinguishing sponge species has implications that go beyond systematic research, affecting ecological studies and management initiatives. When dealing with potentially invasive species, reliable taxonomy is essential. *Hymeniacidon perlevis* is recognized as a morphologically uniform species throughout its distribution. However, given the potential for significant population structure owing to the presumed limited dispersal capacity, we wanted to determine whether morphological uniformity corresponds with genetic uniformity in this geographically widespread species.

In the present study, we employed the analyses of two molecular markers, the mitochondrial cytochrome *c* oxidase subunit I (COI) and ribosomal ITS subunit to validate the taxonomic status of *H. perlevis* along the South African coast, its haplotype diversity, and to evaluate the effects of geographic distance and connectivity in this conspicuous widespread species. While processes of introduction and spread are at this stage speculative, we discuss the most likely scenario and associated management implications.

The type material of *Halichondria caruncula* (sensu Stephens, 1915), *Leucophloeus styliferus* Stephens, 1915 ((*Hymeniacidon stylifera* (Stephens, 1915)) and *Hymeniacidon*

sublittoralis Samaai & Gibbons, 2005 from South Africa were examined. Environmental niche modelling was conducted to investigate the factors that dictate the distribution and the drivers of genetic structure of *H. perlevis* globally.

Materials & Methods

Distribution of *H. perlevis* along the South African intertidal coastline

Hymeniacidon perlevis is the most widely distributed sponge in the intertidal. It grows in a wide range of substrates, such as hard ledges, crevices, flat areas, gullies and pebbles, or sandy substrate in the mid strata of the intertidal zone, but a few specimens have also been recorded at the lower zone and subtidal area (Erpenbeck and Van Soest, 2002; Ackers, Moss & Picton, 2007; Turner, 2020). Some individuals are exposed for hours while others submerged during low and high tides respectively.

Between 2015 and 2017, field surveys were conducted during low tides at 53 intertidal habitats along the South African coast from Port Nolloth (Benguela Current system, 26°44'7.05"S; 15° 5'43.38"E) to Kosi Bay near the Mozambique border (Agulhas Current system, 26°55'46.21"S; 32°52'41.23"E) (Fig. 2; Table S1). Sixty-five (65) specimens of *H. perlevis* were collected from 23 intertidal locations along the South African coast from Port Nolloth on the west coast to the Dwesa/Cwebe MPA on the east coast (Fig. 2; Table S1). The species was found to occur on both exposed and non-exposed regions, and was placed in bags for laboratory processing.

Sample collection

Sponge material was collected from the intertidal rocky shores by removing a representative piece of the animal. Observations on appearance in life, substratum, as well as and habitat depth and type were recorded in situ.Colour photographs were taken in situ and in the laboratory. Upon collection, specimens were stored in 96 % ethanol and processed for histological examinations according to Samaai and Gibbons (2005). Spicule dimensions are given as the mean length (range) × mean width (range) of 20 spicule measurements.

Molecular analyses

DNA was extracted from 54 representative tissue samples across the three major biogeographic provinces using the E.Z.N.A Tissue DNA kit according to the manufacturer's protocol (Omega Bio-Tek). A fragment of the mitochondrial cytochrome *c* oxidase subunit I (COX1) was amplified using primers LCO–1490 (5'–GGT CAA CAA ATC ATA AAG ATA TTG G–3') and HCO–2198 (5'–TAA ACT TCA GGG TGA CCA AAA AAT CA–3') (Folmer et al., 1994). Polymerase chain reactions (PCR) were performed in volumes of 20 µl containing 12.5 µl Taq, 0.5 µl of each primer (10 mM), 1 µl of BSA, 5 µl of DNA template and 5.5 µl H_2O. The cycling profile included an initial denaturation step (3 min at 94°C), 40 cycles of denaturation (30 s at 94°C), annealing (20 s at 45 °C) and extension (1 min at 72 °C), and a final extension step (10 min at 72 °C). The amplified DNA was purified with a PCR Clean-Up Kit according to the manufacturer's protocol. The final DNA product was sequenced in both directions on an Applied Biosystems 3730xl DNA Analyzer (see Teske et al., 2015 for Standard protocols), and the obtained chromatogram was edited using MEGA11: Molecular Evolutionary Genetics Analysis version 11 (Tamura, Stecher & Kumar, 2021). All the sequences was deposited in GenBank (NCBI; Benson et al., 2018) under the accession numbers ON062377–ON062402 (see Table S2).

Alignment, phylogenetic analysis, and Haplotype networks

The raw sequence data of the forward and reverse sequences were trimmed by removing uncertain sites. The forward and reverse sequences were then aligned using ClustalW (Thompson et al., 1994) in MEGA 11 (Tamura, Stecher & Kumar, 2021). Sequences were blasted against GenBank (Sayers et al., 2019) and the maximum score and E-values (Altschul et al., 1990) were used to select closely related specimens. The alignment of COI sequences was checked for the potential occurrence of nuclear pseudogenes using the genetic code for invertebrate mitochondria, and no frame-shift mutations, which would indicate that these sequences originate from a non–functional gene region, were identified. Sequences were compared to published data of *Hymeniacidon* sponges (see Table S3 in Turner, 2020), and thus sequences were jointly analysed with the *Hymeniacidon* date set used by Turner (2020) in MEGA 11 *(*Tamura, Stecher & Kumar, 2021*).* All the South African sequences had a high sequence similarity to *H. perlevis*. Public sequences previously identified as *H. perlevis, H. sinapium,* and *H. heliophila* appear to be from a single species with a global distribution, and all are included herein (Turner, 2020). The dataset compiled by Turner (2020), together with the

South African sequences, was then used in the phylogenetic analysis and haplotype network. To see if the South African samples formed a distinct monophyletic clade in comparison to congeneric samples from other locations, we constructed a phylogenetic tree in MEGA 11 using Maximum Likelihood (ML) with the Tamura-3 parameter (T92), which was selected by the inbuilt model generator. Evolutionary distances were computed employing the Tamura-3 parameter (Tamura, 1992), and support for individual nodes was based on 1000 nonparametric bootstrap estimates (Felsenstein, 1985). The T92 distances were also used to compare levels of genetic differentiation between the sequences generated in this study and the published *Hymeniacidon* sequences (see Table S3 in Turner, 2020).

DnaSP 5.091 was used to evaluate haplotype (h) and nucleotide (π) diversities for individuals collected at the same location (Lourenço et al., 2017). Populations were divided into six groups according to world-wide presence (Turner, 2020; de Voogd et al., 2022). These were East Asia, North America (Pacific), North America (Atlantic), South America (Atlantic), Europe and South Africa. The South African populations were divided into three groups according to the national bioregional classification (see Sink et al., 2018). These include the Namaqua, Southern Benguela and Agulhas ecoregions. To determine how genetic variation is divided between groups, among locations within groups and within locations, the six and four groups described above were designated a priori following Lourenço et al. (2017).

A median-joining haplotype network built in Codon Code v.9 (CodonCode Corporation) was used to establish intra-specific genealogical relationships between groups and the relative frequency of haplotypes. For the haplotype network analysis, the newly collected South African samples were analyzed alongside all sequences from GenBank with high sequence similarity, as compiled by Turner (2020). This included public sequences previously identified as *H. sinapium* and *H. heliophila*, which according to Turner (2020) are part of the single global species complex, *H. perlevis*. Sequences of *H. flavia* are included as outgroups for comparison. Haplotype networks were produced using the minimum spanning method (Bandelt et al., 1999) as implemented in Popart (Leigh & Bryant, 2015). This analysis requires all included sequences to be the same length, so some sequences were trimmed whilst others were excluded. Alignments in the global dataset were 574 bp at cox1 (n=115 sequences); the ITS alignment was 539 bp (n=512). Alignments were longer when newly collected South African data were analyzed alone:

582 bp at cox1 (n=29); 798 bp at ITS (n=11). Sequence alignments were produced in Codon Code v.9 (CodonCode Corporation).

Ecological niche modelling of *H. perlevis* distribution.

To gain a better perspective on the realized distribution of *H. perlevis* globally, ensemble species distribution modelling was applied.

Occurrence data. For this purpose, occurrence/encounter data from multiple sources was complied, including OBIS records, GenBank, and local South African observations. Because the data are occurrence/encounter data, background absence (pseudo-absence) data are required to apply standard correlative distributional models. Pseudo-absences were generated at random within the studies gridded spatial domain, with the thin layer of coastal area being globally generated. Given the environmental layers resolution (5 × 5 NM grid cells), the first two adjacent grid cells were considered. The number of pseudo-absences generated were determined using the recommendations of Barbet-Masin et al. (2012), implemented in the R package SSDM (Shmitt et al. 2017), which was used in this study for ensemble species distribution modeling. Ensemble modeling was performed both with and without spatial thinning. Given that the encounter data are not globally uniform, spatial thinning, which is already implemented in the SSDM package, was used to deal with spatial bias (to reduce spatial bias due to non-random sampling while keeping most of the information).

Niche modelling. Multiple correlative statistical models are widely used to model the distributions of many taxa. The majority of these widely used models are already included in the SSDM package. For the ensemble modeling of the distribution of *H. perlevis*, eight correlative statistical models were considered: Generalized Linear Model (GLM), Generalized Additive Model (GAM), Support Vector Machine (SVM), Classification Tree Analysis (CTA), Generalized Boosted Model (GBM), Random Forest (RF), Multivariate Adaptive Regression Spline (MARS), and Artificial Neural Network (ANN). Appendix S1 contains a brief discussion of each of the eight distribution models. These models were trained on a randomly selected portion of the data (70%) and their prediction performance was evaluated using the hold-out set. Each model was fitted and evaluated four times to account for sources of variability due to random selection of training and evaluation sets as well as random selection of pseudo-absences. When analyzing classification models, multiple measures of performance can be used. For the

purposes of this study, Kappa and AUC (Area Under the Curve) were used. AUC usually has a value in the range of 0.5 to 1.

Models with AUCs of 0.5 are generally considered random classifiers, while those with values between 0.7 and 0.8 are considered fair classifiers, and those with values close to 1 are considered excellent classifiers (Kleinbaum & Klein, 2010). The inclusion of individual distribution models into the ensemble distribution modeling was based on whether the model had an AUC value greater than 0.7. To generate the ensemble species distribution map, all models with AUCs greater than 0.7 were pooled by weighting their predicted probability of occurrence by their AUC. The uncertainty map was also computed primarily to identify regions of high agreement and low agreement among the models considered, which correspond to low and high uncertainty regions, respectively. Uncertainty was computed as cell by cell variance in the predicted probability of occurrence by the models included in the ensemble. The response curves (partial effects) of each of the variables considered was generated by predicting the probability of occurrence for the variable of interest while keeping the remaining variables at their mean. This was done for each of the eight models considered (Appendix S1).

The intertidal area was delimited by extracting the coastal cells covering a range from −2 to 1 m from the General Bathymetric Chart of the Oceans (GEBCO) gridded bathymetric data set with a spatial resolution of 30 arc-seconds (http://www.gebco.net/).

Variable importance was computed on the holdout set. The amount of correlation changes between predicted values before and after permuting (reshuffling) a variable was used to measure its importance (expressed in percentage).

$$I_v = 1 - Cor(P_f, Pv)$$

where I_v is index of importance of a variable, Cor is correlation coefficient, P_f is prediction from the full model, P_v is prediction after permuting/reshuffling the variable v. Partial effect of each of the predictor were computed by predicting the response variable for the variable of interest while holding the other predictors at their mean.

Environmental variables. BIO-ORACLE was used to download global and readily available environmental layers. The environmental layers considered in this study considered the minimum, maximum, mean, and range of surface temperature, surface salinity, and surface current velocity. Given the limitations of the environmental layers resolution (5 × 5nm grid) and the fact that we are dealing with intertidal/coastal invertebrates, only grid cells within 10 km of

the coastline were retained. The environmental variables were checked for multi-collinearity using the variance inflation factor (VIF) before the distribution of *H. perlevis* was modelled.

A VIF value of 1 indicates an absence of multi-collinearity, but larger values typically indicate the presence of a problem. Variables with VIF values > 5 are generally considered to be linearly related, and in the context of regression, their variance of the estimated parameters will be large, and its parameter will be poorly estimated (Hay-Jahans, 2011). Variables having VIF greater than 5 were thus excluded from this analysis. The final set of variables retained were: mean surface temperature, range surface temperature, mean surface salinity, range surface salinity, and mean surface current velocity. Appendix S1 shows the layers of environmental variables used in the distribution modelling.

The following model was fitted to model occurrence of the sponges.

Model formula

$$occurrence \quad sponge_{occ} \sim T_{mean} + T_{range} + S_{mean} + S_{range} + V_{mean}$$

where $sponge_{occ}$ is the occurrence is of *Hymeniacidon perlevis*; T_{mean} and T_{range} are the mean and range of coastal surface temperature respectively; S_{mean} and S_{range} are the mean and range of coastal surface salinity respectively; V_{mean} is the mean coastal surface current velocity.

All the analysis, visualization and report generation were done in R (R Core Team, 2021). Multiple R packages were utilized for data processing, visualization, analysis, and summary of results including (Alathea, 2015; Allaire et al., 2021; Henry and Wickham, 2020; Robinson et al., 2022; Wickham et al., 2021b, 2021a; Xie, 2021; R-lubridate?).

Material and acquisition

All recently collected voucher samples are housed at IZIKO Museum, Cape Town, South Africa under museum numbers SAMC-A091444–SAMC-A091463; MB-A094583–MB-A094599; MB-A094600 - MB-A094614 (Table S3). Toufiek Samaai was granted permission to collect specimens during his various field excursions by the Department of Forestry, Fisheries, and Environment under Research Permits RES2014/DEA - RES2017/DEA.

Results

Study area

The South African coastline covers a wide range of very distinct climatic and oceanic conditions that can be divided into three major biogeographic provinces (Stephenson & Stephenson 1972; Emanuel et al., 1992; Turpie et al., 2000; Sink et al., 2019). These are the subtropical east coast, the warm-temperate south coast, and the cool-temperate west coast (Emanuel et al., 1992; Sink et al., 2019). Interestingly, the shores are characterised by sharp environmental clines, in particular the contrasts in temperature (Smit et al., 2013), productivity and dissolved oxygen, associated with abrupt species distributional changes (Emanuel et al., 1992; Awad et al., 2002, Sink et al., 2019). The west coast of South Africa is permanently affected by the cold waters of the Benguela upwelling system, whereas the south flowing Agulhas current transports warm water along the east and south coasts of South Africa (Lutjeharms et al., 2000; Lutjeharms, 2006; Smit et al., 2013).

South Africa is an ideal region to study the effects of dispersal barriers and environmental gradients on species distribution and genetic patterns (Emanuel et al., 1992; Awad et al., 2002). Cape Point and Cape Agulhas connects the south-west coast of South Africa, represents a geographical break for several cool temperate and warm-temperate biota and is a driver of genetic differentiation (Teske et al., 2011). The region between these sites is considered a transition zone between the cool temperate west and the warm temperate south coasts. Biogeographical breaks on the south-east coast, at the disjunction between warm-temperate and subtropical biota, have been difficult to define because their exact locations differ considerably for different species (Teske et al., 2011). The continental shelf in this region gradually widens from north to south, deflecting the warm Agulhas Current away from the coast, limiting its influence on coastal biota. The northernmost breaks in this region have been identified on the Central Wild Coast (Transkei region in the region of Mbashe) and the southernmost breaks were reported near Algoa Bay (Teske et al., 2011).

Emanuel *et al.* (1992) proposed that the division between the south-east coast was south of Coffee Bay at Mbashe and recognised an additional break at Durban, similar to the location of the eastern extent of the overlap region transitioning between the original south-east coast provinces identified by Stephenson & Stephenson (1948). Emanuel *et al.* (1992) also found no further biogeographic breaks north of Durban to at least as far as Ponta da Barra Falsa in Mozambique. However, Bolton *et al.* (2004) argued against the existence of a distinct east coast

province because the marine flora reflects an eastwardly decreasing number of temperate South Coast species, replaced largely by tropical Indo-West Pacific species.

Inshore upwelling areas off the south coast's Cape St Francis, Still Bay, Tsitsikamma, Algoa Bay and Port Alfred may potentially influence species range extensions and the size structure of habitat forming species, such as mussels, macroalgae and sponges (Mead et al., 2013; Lourenço et al., 2017). Additionally, the Agulhas Bank has also experienced expansions of cold-water species, particularly along Betty's Bay to Cape Agulhas as a response to SST changes (as much as −1.5º C since the 1980s) (Mead et al., 2013). For example, southeast extension of kelp beds, rock lobster, cape sea urchin and sardines have exhibited major distributional expansions along south coasts linked to rates of SST cooling over the last two decades (a maximum of −0.5º C per decade) (Mead et al., 2013). Mean annual air temperature in South Africa also increased by 0.13 ºC per decade between 1960 and 2003, potentially influencing intertidal ecosystems such as rocky shores (Mead et al., 2013).

Distribution of *Hymeniacidon perlevis* along the south African intertidal region

Hymeniacidon perlevis was found at 23 of the 53 locations sampled (Fig. 2; Table S1). The easternmost location where *H. perlevis* was found was the Dwesa/Cwebe MPA on the east coast. A sample was also collected at Grosse Bucht, which is south of Lüderitz (Namibia). Individuals of this species were not found north of Dwesa/Cwebe, replaced in abundance by the encrusting blood-red sponge *Tedania* cf. *scotiae* (Fig. 3M), a species with similar distribution to *H. perlevis*, indicating the possible existence of suitable habitat for *H. perlevis* in the Natal intertidal ecoregion. However, H. perlevis, does not occur further north of Dwesa/Cwebe. *Tedania* cf. *anhalens* (Fig 3P) occurs in the Delagoa ecoregion.

Hymeniacidon perlevis dominates the intertidal shores from the cool temperate province (minimum SST ranging 12.2–14.8; winter SST 13–15) (Demarcq, Barlow and Shillington, 2003; Smit et al., 2013) on the west coast to the warm temperate province (minimum SST ranging 14.5–18.7) on the south/southeast coast; winter SST 15–19, (Demarcq, Barlow and Shillington, 2003; Smit et al., 2013) but it is absent from the subtropical province of the Agulhas system north of Dwesa/Cwebe.

In only one location (Strand) we found *H. perlevis* covered by sediment, with surface projections extending beyond the sandy layer (Fig. 3G). Apart from this, *H. perlevis* was also

found in places along the Cape Peninsula with high nutrient concentration (Villiers de S., 2017).

Hymeniacidon perlevis was also described from the kelp forest off Bettys Bay at a depth of 15 m while Stephenson (1915) recorded the species from 25 m depth on the west coast of South Africa. Turner (2020) found *H. perlevis* in California kelp beds. Samaai et al. (2005) described a subtidal *Hymeniacidon* species from Vulcan Rock (Cape peninsula west coast) as *Hymeniacidon sublittoralis* Samaai & Gibbons, 2005.

Genetic analysis *Hymeniacidon perlevis* along the South African intertidal region and globally.

No amplification product for *Halichondria caruncula* (Bowerbank, 1858) sensu Stevens (1915), *Hymeniacidon stylifera* (Stephens, 1915) and *Hymeniacidon sublittoralis* Samaai & Gibbons, 2005 from South Africa could be obtained. Partial COI sequences of 29 specimens of *H. perlevis* (Table S2) were obtained resulting in an alignment length of 691 base pairs with two different haplotypes and a low intraspecific genetic distance (0.0017, +- SD or SE estimate) and a slightly high haplotype diversity (0.517, +-SD or SE estimate). Closer examination of the network reveals that the two haplotypes differ by one mutational change. The genetic structure of *H. perlevis* revealed a possible divergence on the south-east coast, forming a western and an eastern lineage. Specimens from the south coast (Knysna, Tsitsikamma) clustered with those from 500 km to the east, rather than those from 500 km to the west, in the maximum likelihood tree (Fig. S1). Sequences could not be obtained from specimens collected from sites between Bettys Bay and Knysna, particularly near Cape Agulhas, where the two oceans meet.

BLAST results revealed 99–100% similarity match when comparing South African *H. perlevis* sequences to *H. perlevis* and *H. synapium* from other worldwide regions (Portugal, California, etc.). This was further corroborated by phylogenetic reconstruction as sequences of *H. perlevis* from South Africa grouped with those from other regions and *H. synapium* in the maximum likelihood tree (Fig. S2), with weak maximum likelihood support (prob val. 43). The *H. perlev*is sequences further showed about 97% similarity to *H. flavia* Sim & Lee, 2003 (EF217333.1, Korea), which is the most closely related outgroup species (Fig. S2) (Park et al., 2007).

The reconstruction of a median-joining haplotype network of *H. perlevis* from South Africa revealed a single clade, with no genetic divergence between a priori expected groups (Fig.

4). The star-shaped network revealed two dominant haplotypes widespread across the three regions. The shared haplotypes differed from the dominant haplotype by one or two mutational steps. Regardless of the geographic distance between groups, haplotypes were shared (i.e., between Port Nolloth and Dwesa or Cape Point and Bettys Bay).

The inclusion of previously published *Hymeniacidon* sequences from GenBank (see Turner, 2020 Table S3; see Ngwakum, 2021 Table 1; see Table S2) revealed that for the ITS dataset, four of the South African samples are identical (Fig. 4) to the most common haplotype, found in a sample from Northern California, five samples from Korea, and many samples from Japan (Fig. 4). The seven South African samples differ from this most common haplotype by a single base pair (0.19% sequence divergence). Consistent with a previous analysis, the genetic variation within the sample of *H. flavia* is similar to the genetic variation in *H. perlevis*, despite the entire *H. flavia* sample being from Japan and Korea alone (Fig. 4; see also Fig. 2 in Turner, 2020). When the South African ITS samples are analyzed alone, a longer alignment is possible (798 bp), and three variable sites are present (Fig. 4). One of these differentiates samples from Agulhas from the other regions. Only two haplotypes, differing by a single base pair, are present in South African samples in the COI haplotype network. Nearly half (14/29) possess the same haplotype sequence as samples collected in the Caribbean, Brazil, Portugal, Turkey, Korea, and California. A close genealogical relationship among haplotypes was observed.

Subsequently, we employed a COI haplotype analysis on the South African specimens comparing them to the global *H. perlevis* COI sequences from GenBank (Turner, 2020). Results from the 134 sequences revealed a total number of 16 distinct COI haplotypes, with a diversity of 0.225 (+- SD or SE estimate), which is relatively low. According to the results, all South African sequences belong to the first haplotype, which also includes *Hymeniacidon* from Portugal, Argentina, Turkey, and other countries (Fig. 5). The others represents singleton haplotypes since they only contain one individual and group among specimens of *H. sinapium* from China and *Hymeniacidon heliophila* (Wilson, 1911) from the Caribbean.

The ITS sequences revealed four haplotypes for the South African specimens' samples with a single base change between the sequences. This ITS was also used to explore the global relationship of *H. perlevis* (Fig. 5). The recovered ITS topology clustered all South African *Hymeniacidon* with sequences from *H. perlevis and H. sinapium* in a single monophyletic clade. All specimens of *Hymeniacidon* from South Africa shared the same ITS haplotype, which was

proved to be identical, for instance, to GenBank sequences of *H. perlevis* (AB250766.1), *H. heliophila* (AB250764.1) (U65485.1) and *H. sinapium* (AB250765.1) from Japan.

Environmental niche modelling.

Hymeniacidon perlevis is unusual in that it has a very wide geographic distribution ranging from the Northern Hemisphere to Argentina, as well as South Africa to the middle latitudes of New Zealand. Visual exploration of occurrence data for *H. perlevis* from the three sources, OBIS, GenBank, and local South African coastal monitoring, are shown in Figure 1. As can be seen in Figure 1, most of the occurrence records are from coastal area around the UK and South Africa, with the remaining records from different parts of the globe, such as harbours and inlets.

Figure 6 depicts the predicted distribution, as well as the probability of occurrence, of *H. perlevis* from each of the eight models and the ensemble (Appendix S1). The ensemble prediction on the raw and thinned data is shown in detail in Figure 6. The projected likelihood of occurrence was not significantly different for models based on thinning or raw occurrence data when seen visually. The ensemble produced with the best models resulted in an accurate overall description of *H. perlevis* distribution, including its expanding front (Fig. 6).

Along South Africa, the niche model predicted a distribution into Namibia and in South America from southern Chile into northern Peru (Fig. 6). In addition, the prediction indicated that suitable habitat could potentially be found along southern Australia and Namibia. While the probability of *H. perlevis* being present in the Mediterranean and British Columbia shores was high, towards Northeast America the predicted likelihood decreased. Surprisingly, no suitable habitat was detected along the tropical West African coast, the Indo-Pacific region, Caribbean, the Arabian Peninsula or India. Mean surface temperature was the most important predictor of the distribution of *H. perlevis* globally (Appendix S1), followed by the range of surface temperature. Most of the models performed reasonably well with *AUC* mostly above 0.8.

Taxonomy - Species description

Systematic information with detailed morphological and spicule descriptions, and with DNA-barcoding remarks are provided below. The classification follows Morrow & Cárdenas (2015).

Phylum Porifera Grant

Class Demospongiae Sollas

Subclass Heteroscleromorpha Cárdenas, Perez & Boury–Esnault

Order Suberitida Chombard & Boury–Esnault

Family Halichondriidae Gray

Genus ***Hymeniacidon*** **Bowerbank, 1858**

Type species. *Hymeniacidon caruncula* Bowerbank, 1859: 286 (by subsequent designation; Bowerbank, 1864: 191) (this is considered a junior synonym of *Hymeniacidon perlevis* (Montagu, 1818: 86).

Hymeniacidon perlevis **(Montagu, 1818)**

(Fig. 7AC; Table 1)

Material examined. Table S3

Other material examined. *Hymeniacidon perlevis* voucher specimens. SAM-H4904 (Ts 305), Jacobs Bay, near Saldanha Bay (32 31'S, 17 30'E), depth 3–5 m, collected by T. Samaai, 20 October 1997. Ts 329, Ts 331, Ts 337, Ts 338, Ts 343c, Elands Bay (32 20'S, 18 20'E), depth 3–6 m, collected by T. Samaai, 15 November 1997. Ts 359, Ts 370, Ts 381, Ts 391, Groenrivier (30 29'S, 17 20'E), depth 3 m. Collected by T. Samaai, 20 December 1997.

Hymeniacidon caruncula (Bowerbank, 1858) sensu Stevens (1915). NMSZ 1921.143.1443. A fragment removed for loan. Station 479, False Bay shore 6th May 1904; Station 482, Saldanha Bay shore, 19 May 1904.

Leucophloeus stylifera Stephens, 1915. Syntype NMSZ 1921.143.1443. Two fragment removed for loan. Station 482, Saldanha Bay shore, 19th May 1904; Station 483, Entrance to Saldanha Bay, 45 m, 21 May 1904.

Hymeniacidon sublittoralis Samaai & Gibbons, 2005. Holotype. SAM-4903 (Ts 212), Vulcan Rock (34 04'S, 18 18'E), depth 27 m, collected by P. Coetzee, 24 April 1996.

Description. A thin or thickly encrusting (Fig. 7A) to cushion-like sponge that varies greatly in form (Fig. 2), Diameter ranging from 5 cm long × 3 cm long × 4 cm thick to 14. 5cm long × 8 cm wide × 6 cm thick; with processes of 1–4 mm high, 1–1.5 mm wide. In areas of considerable wave exposure this species is encrusting and smooth. In sheltered, or moderately exposed localities, *H. perlevis* has erect processes that arise from a basal mat. Surface variable, may be smooth and tuberculate, thrown into irregular folds, or covered with digitate processes.

Oscules scattered, level with surface or elevated on low cones or on digitate processes, 0.5–1.5mm in diameter. Texture firm, soft, fleshy, but compact and compressible. Colour in situ variable (Fig. 2): intertidal forms bright orange, subtidal forms yellow orange; choanosome bright orange, in preservative pale yellow or white, choanosome bright orange or white. Colour changes on sampling - the sponge might turn patchy dark brown or appear greenish (Fig. 2D).

Skeleton. The choanosomal skeleton, especially in the deeper regions, composed of a confused, disordered mass of styles, not organized into tracts (Fig. 7C). Towards the surface, tracts become ill-defined and with ascending fibres, ~200 μm wide, with no separation between the primary and secondary tracts. The ascending tracts do not branch at the surface to form spicule brushes and tend to be vertically arranged. Numerous loose interstitial spicules. Large canals are present. The ectosomal skeleton consists of a dense tangential layer of spicules, ~200–500 μm thick (Fig. 7C). Spongin scarce.

Spicules. Megascleres (Fig. 7B; Table 1): styles smooth, straight, or slightly curved, thickest centrally, 250 (155–337) × 7 (7) μm, n = 20. Microscleres: absent.

Habitat and distribution. Found on the rocky intertidal and shallow subtidal. Often exposed to direct sunlight. Depth range 0–15 m.

Status. Invasive

DNA barcodes. 691bp fragment of the universal mitochondrial cytochrome *c* oxidase subunit 1 gene, primer pair: LCO1490 and HCO2198 (Folmer et al., 1994). GenBank accession numbers ON062377–ON062402 (see Table S2).

Remarks. Comparisons of the morphological features of the intertidal sponge *H. perlevis* with *Hymeniacidon sublittoralis* Samaai & Gibbons, 2005, *Hymeniacidon stylifera* (Stephens, 1915) and *Hymeniacidon caruncula* Bowerbank, 1858 sensu Stevens (1915) showed that it is similar to *H. caruncula* but distinct from *H. sublittoralis* and *H. stylifera* in spicule shape, spicule dimensions, external morphology, and coloration (Stable S4). Live specimens of *H. perlevis* have distinct colour patterns being different shades of orange depending on geographical location (Fig. 2). Though this species can be blood red in other regions, in the current study, intertidal encrusting sponges of that colour were species of the genus *Tedania (*Fig. 2).

The spicule size range of South African *H. perlevis* overlaps with specimens from Wales, Korea and Ireland (Fig. 8). A large spicule size range is found for the South African west coast and Wales specimens (see Fig. 8). California specimens have a smaller spicule range, with

lengths very similar to New Zealand and Argentina (see Fig. 8). No spicule lengths are available for specimens from Portugal, Spain, France, China, Black Sea etc. The South African specimens conform to all previous descriptions in terms of morphology and spicule complements, as well as in habit (Bergquist, 1970; Ackers et al., 1992).

Morphologically, this species is distinct from *H. sublittoralis*. *Hymeniacidon sublittoralis* is " A thick, massive, erect, amorphous sponge, with numerous papillate processes that vary greatly in length. Surface smooth with various ridge-like structures, finely hispid and colour in situ yellow. Styles are large and thick with heads slightly subtylote, 394 (255–601) × 14 (14) μm (Samaai and Gibbons, 2005).

Discussion

Hymeniacidon species, like *Halichondria* and *Cliona* spp. (de Paula et al., 2012), exhibit a high degree of morphological similarity, making it difficult to recognize them solely by morphological characters. Traditional morphological features like spicule shape and size, as well as their skeletal arrangement, are insufficient to distinguish species in this likely complex.

The South African sequences of *H. perlevis* were compared against those of *H. perlevis, H. sinapium* and *Hymeniacidon heliophila* (Wilson, 1911) from Europe, South America and North America reported in Turner (2020). These data conclusively document the presence of *H. perlevis* in South Africa as well as assign the species to a relatively large geographic range along the South African coast.

The extensive distribution of *H. perlevis* across several biogeographic areas suggests that the observed pattern cannot be maintained by natural dispersal and gene flow at such a broad scale (e.g., some South African individuals were genetically identical to individuals from California); the low molecular divergence associated with their extensive distribution around the world (Turner, 2020) are typical features of species introduced on a worldwide scale. As a result, human-mediated gene flow is likely to play an important role in population connection and spread (Mead et al., 2013). Because larvae have a very limited chance of survival due to their short free-swimming phase, the most likely vectors for this species are the transfer of adult colonies in ship hulls or larvae transported in ballast water (Duran, Pascual & Turon, 2004).

The worldwide introduction and subsequent spread of *H. perlevis* (Turner, 2020), and its success in adapting to new environments, likely reflects the species' high adaptability. There is

586 evidence of several oceanic barriers along the South African rocky intertidal region (Emanuel,
587 1992; Samaai, 2006; Teske et al., 2011; Sink et al., 2019), which have the potential to reduce
588 connectivity between populations to a high degree, as previously described for *Perna perna*
589 (Lourenço et al., 2017) and several other species (Turpie et al., 2000; Awad et al., 2002; Samaai,
590 2006). However, our results suggest a lack of genetic difference across *H. perlevis* populations
591 across the study region. Furthermore, the niche modeling approach highlights the importance of
592 sea surface temperature (SST) in shaping the distributional range of *H. perlevis* along temporal
593 regions along the southern African coast. We identified two to four haplotypes for the COI and
594 ITS genes surveyed across the distribution of *H. perlevis*, respectively.
595 *Hymeniacidon perlevis* dominates the intertidal shores from the cool temperate province
596 (minimum SST ranging 12.2–14.8 ºC; winter SST 13–15 ºC) (Demarcq, Barlow & Shillington,
597 2003) on the west coast to the warm temperate province (minimum SST ranging 14.5–18.7 ºC)
598 on the south/south east coast; winter SST 15–19 ºC) (Demarcq, Barlow and Shillington, 2003)
599 but it is absent north of Dwesa/Cwebe and the subtropical province of the Agulhas system. The
600 subtropical and tropical waters of the east coast may be outside the optimal temperature range of
601 *H. perlevis*. Having said this, the temperature range between Mbashe and Port St Johns [warm
602 temperate] is like that of Dwesa/Cwebe. Turpie et al. (2002) also identified the whole of the
603 Transkei region in the Eastern Cape as being closer to the south coast sections than to those
604 further north. *Hymeniacidon perlevis* expansion on the south coast over the last 80 years
605 (Stephenson, 1938, 1944 & 1948; Day, 1974) has virtually ceased at Dwesa/Cwebe, and it may
606 have reached its biogeographic limit, as in the case of *Mytilus galloprovincialis* (Zardi et al.,
607 2007; Lourenço et al., 2017).
608 This limit could be explained that the area between Algoa Bay and Dwesa/Cwebe is a
609 transitional zone for species overlap and that *H. perlevis* invasion in South Africa conforms to an
610 antitropical distribution pattern typically found for *M. galloprovincialis* (Zardi et al., 2007). This
611 may also indicate an oceanographic barrier to larval dispersal or selection driven by sharp
612 gradients in environmental conditions at Dwesa/Cwebe. However, the precise temperature limits
613 of *H. perlevis* distribution are unknown. Nevertheless, some indication of its preferred
614 temperature range can be gleaned from the species' global distribution predictive pattern (Fig. 6).
615 The annual mean temperature of its native range in Devon, southwest England is close to
616 11–12 °C (Anonymous), whereas long term temperature means over the distributional range of

introduced *H. perlevis* in California are 17 °C and in Japan between 10 °C and 15 °C (Kado, 2003). Similarly, the temperature range in Patagonia is between 9–16 °C and in Portugal is on average 15.3 °C. This suggests that *H. perlevis* is a cool/warm temperate species that is unlikely to extend its distribution eastwards beyond its current temperature range along the west and south-east coasts of South Africa to average temperature exceeding 20 °C**.**

The area from Cape Point and Cape Agulhas is an interface region where several cold- and warm-water species reach their southern or western distributional limits (Emanual, 1992; Sink et al., 2019), with several new distributional patterns resulting from range expansions and contractions east of Cape Peninsula being attributed to decreases in average seawater temperatures (e.g. kelp, rock lobster, and macroalgae) (Bolton et al., 2012; Blamey & Branch, 2012; Mead et al., 2013; Reimers et al., 2014; Sink et al., 2019).

The low dispersal ability of pelagic larvae and high genetic diversity among *H. perlevis* sampled in South Africa using the mitochondrial COI and nuclear ITS, together with its spatial distribution and relationships of haplotypes in the median-joining network, indicates a lack of spatial genetic structure and reveal that *H. perlevis* is a single population that was probably introduced via centuries of trans-Atlantic and Indo Pacific shipping. This requires further exploration with more rapidly evolving markers, such as microsatellites and SNPs. The latter have the potential to elucidate structure not detected by mtDNA or even microsatellites (e.g., Gouws et al., 2020).

At a large scale, no strong genetic division between geographically defined groups of populations globally are highlighted by mitochondrial (COI) and nuclear (ITS) markers, though this appears to reflect the evolutionary history of the species (Turner, 2020). Globally, *H. perlevis* showed a large distribution across several biogeographic areas; the observed pattern cannot be maintained by natural dispersal and gene flow at such a broad scale (e.g., some South African individuals were genetically identical to individuals from California); the low molecular divergence associated with their extensive distribution around the world (Turner, 2020) is a typical feature of species introduced on a global scale.

A low level of genetic variation at partial COI sequences (p= 0.0006) has been reported among *Crambe crambe* sponges separated by a geographic distance up to 3 000 km from the Mediterranean and the Atlantic coast *(*Duran et al., 2004*).* Even less mitochondrial gene variability was observed among the sponge *Astrosclera willeyana* sensu lato (p= 0.00049)

irrespective of the wide geographical coverage (Wörheide, 2006). By comparison, our study showed a similar low genetic diversity for *H. perlevis* separated by a geographic distance of up to 2 500 km (COI, 0.00017) revealing considerable stability in this intertidal sponge species. Apart from this, the success of *H. perlevis* could also be due to its high rate of growth and survival, dispersal by means of fragmentation and production of bioactive secondary metabolites (Gaino et al., 2010; Won et al., 2017).

Widespread geographic distribution of sponges is rare due to the presence of cryptic species (Carballo et al., 2013). Carballo (2013) showed the presence of several widespread species along the Pacific Ocean because of anthropogenic activities such as shipping. In an increasingly connected world, our research highlights the importance of understanding connectivity across various biogeographic provinces to predict where invasive species are likely to occur. The successful invasions of *H. perlevis* in South Africa, Argentina, North America, Europe, and Japan does not suggest that this species may pose a threat to intertidal communities in cool/warm temperate areas. There is no evidence in South Africa that *H. perlevis* displaced any species in low energy environments, like *M. galloprovincialis*. The findings of this study emphasise that similar environmental conditions appear to facilitate successful marine introductions.

Conclusions

This study builds on a global genetics dataset that confirms *H. perlevis* as an invasive species outside of its natural environment, having been introduced by shipping and other human-mediated activities. From Port Nolloth (including Lüderitz in Namibia) on the west coast to Dwesa on the east coast, *H. perlevis* has expanded and become a very widespread component of low/mid-shore intertidal rocky shore communities, where it provides a habitat for numerous other species that colonize it. There is no indication, however, that the species is displacing native species. Increased subtidal surveys for *H. perlevis* should be conducted to determine its density and whether it is higher in the subtidal zones than in the intertidal zones, which would provide a better indication of the invasion's severity.

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Author contributions

TS & S.E.K funded the research. T.S, BN, RP conceived the study and carried out the sampling. T.S, BN & RP conducted the barcoding lab work. TT, TS and JK analysed the genetics data and TS identified all the samples as *H. perlevis*. SDM analysis were performed by DY. The main manuscript was prepared by TS, with assistance from all other authors. All authors reviewed and edited the manuscript.

Ethics and permits

We acknowledge that it is understood that with the submission of this article the authors have complied with the institutional and/or national policies governing the humane and ethical treatment of the experimental subjects and that we are willing to share the original data and materials if so requested. No ethics clearance was required for the survey and collection of intertidal sponges. The national collection permits were obtained from the Department of Forestry, Fisheries and Environment, Oceans and Coasts Branch.

Competing interests

The authors declare no competing interests.

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Figure 1

Global occurrence records of *Hymeniacidon perlevis*

Global occurrence records of *Hymeniacidon perlevis*

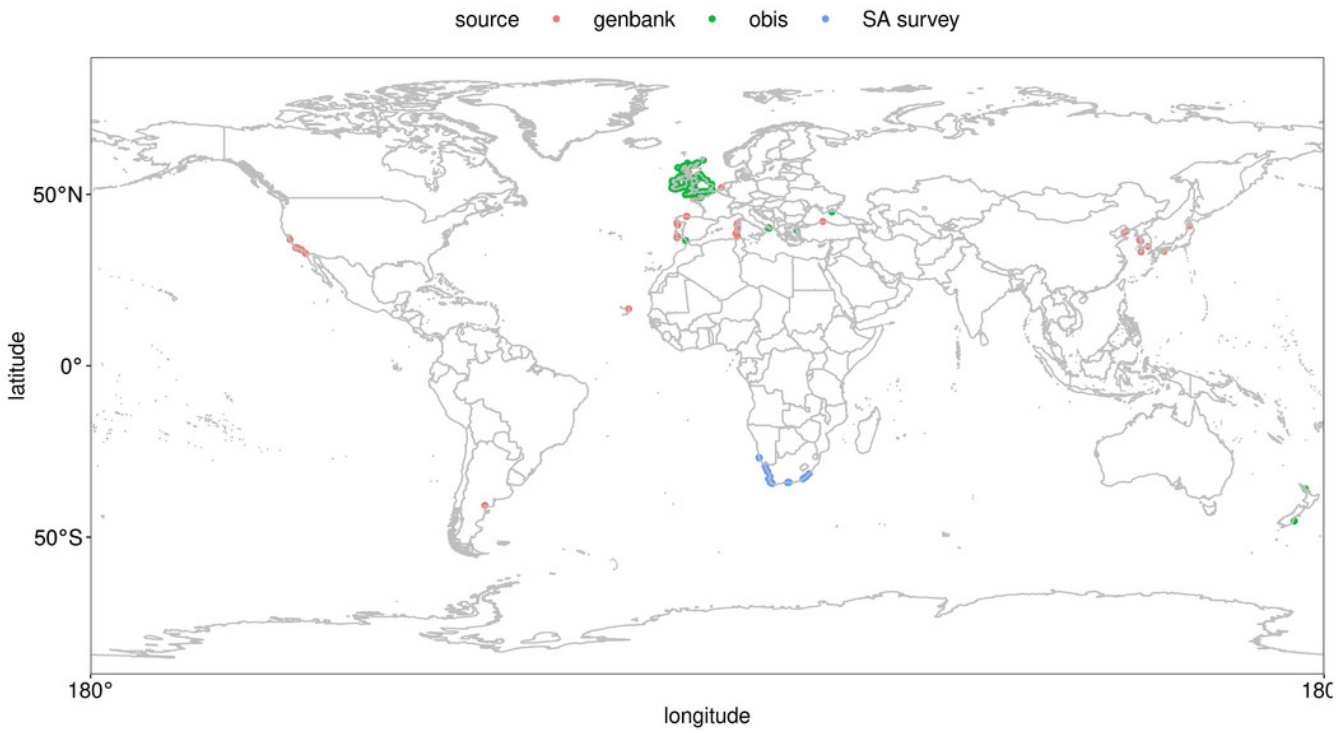

Figure 2

H. perlevis range along the South African and Namibian coastline.

H. perlevis range along the South African and Namibian coastline. Presence and absence of *H. perlevis* marked by blue and red dots respectively. White dots represent surveyed sites where no *H. perlevis* was found. Surveyed locations are described in Supplementary Table 1, from west to east. Arrow indicates north. The map was created using the open source software R.

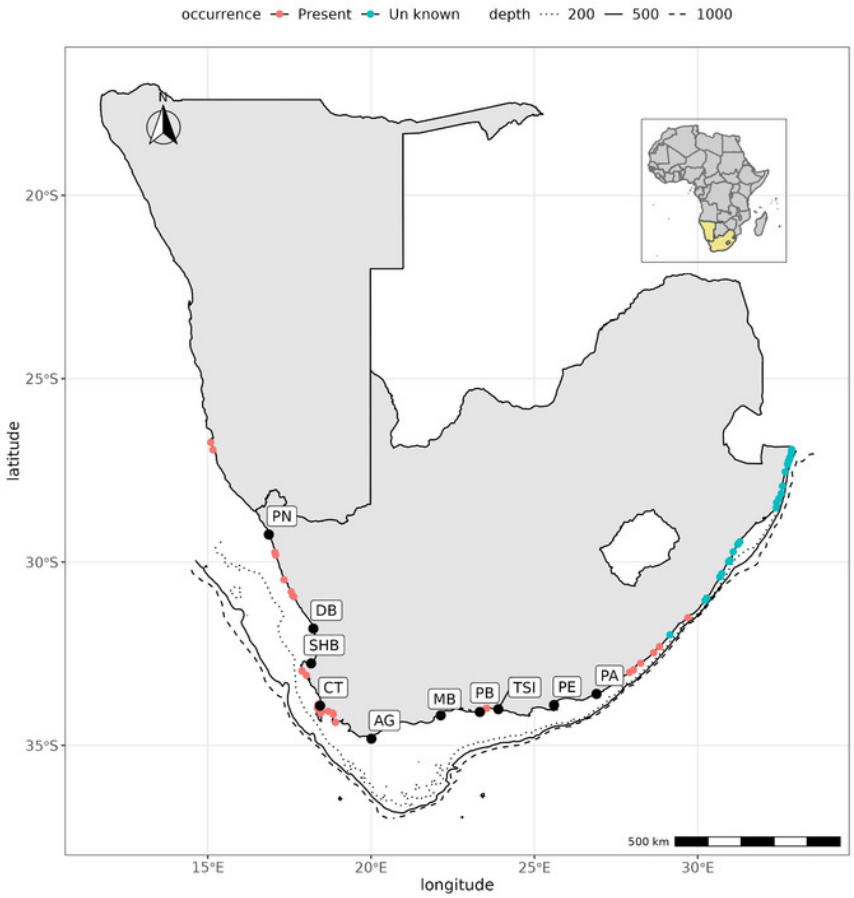

Figure 3

Images of *Hymeniacidon perlevis* collected from different intertidal rocky shores along the South African coastline.

Images of *Hymeniacidon perlevis* collected from different intertidal rocky shores along the South African coastline. A. Groenrivier mund (photo credit Prof. George Branch); B. Moon Bay; C. Elands Bay (photo credit Prof. George Branch); D. Jacobs Bay; E. Springfontein (photo credit Prof. George Branch); F. Cape Peninsula Greenpoint; G. Stand, H. Kommetjie (photo credit Prof. George Branch); I. Bettys Bay; J. Tsitsikamma; K. Dwesa (photo credit Prof. George Branch); *Tedania anhalens* from L. Dwesa, M. Coffee Bay, N. Hluleka (photo credit Prof. George Branch), O. Port St Johns and P. Sodwana Bay.


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Figure 4

Minimum-spanning genotype networks for two loci for South African samples. Samples are coded by bioregion.

Minimum-spanning genotype networks for two loci for South African samples. Samples are coded by bioregion.

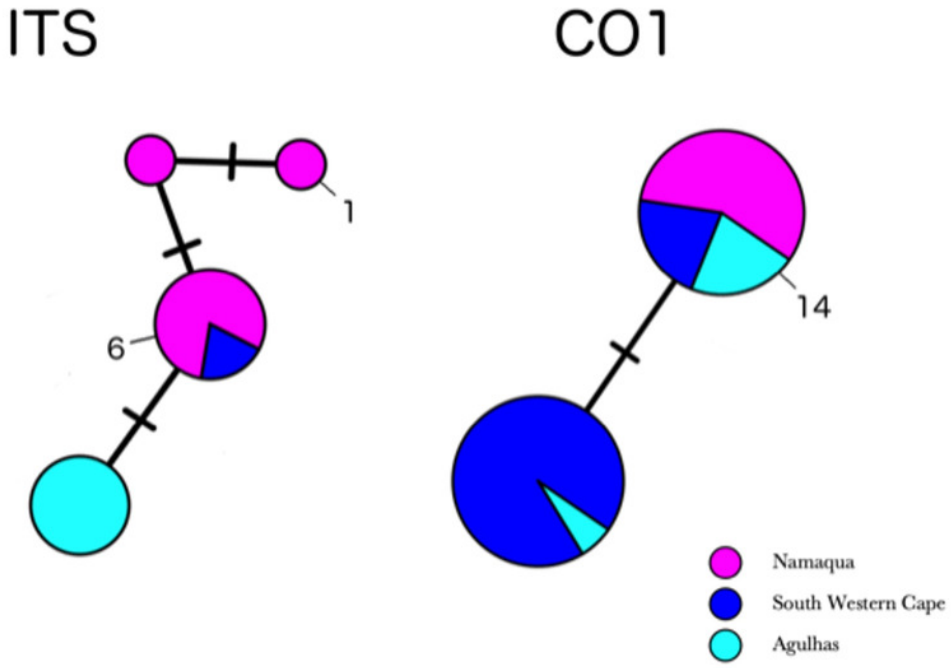

Figure 5

Minimum-spanning genotype networks for two loci.

Minimum-spanning genotype networks for two loci. Samples are coded by collection location, regardless of whether they were identified as *H. perlevis, H. sinapium,* or *H. heliophila*. Closely related *H. flavia* are shown for comparison where available; all data for this species is from Japan and Korea.

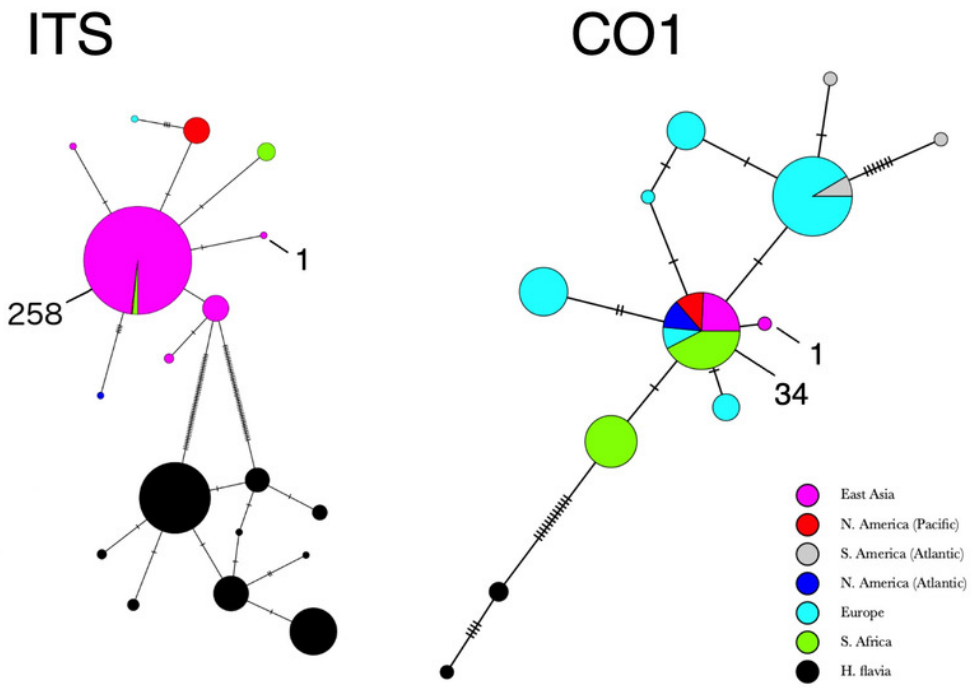

Figure 6

Predicted global distribution for *Hymeniacidon perlevis*

Predicted global distribution for the orange/red encrusting sponge *Hymeniacidon perlevis* derived by averaging an ensemble of presence-absence algorithms. Shown for the thinned and raw data.

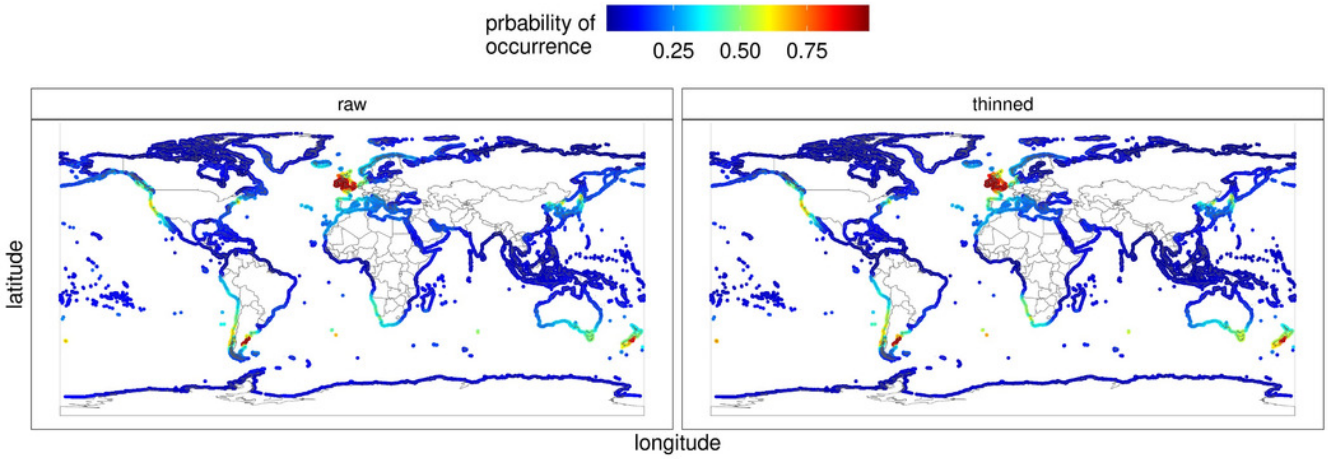

Figure 7

Hymeniacidon perlevis photomicrograph and in situ image

Hymeniacidon perlevis, A. in situ; B. Photomicrograph of spicule compliment, styles; C. Transverse histological section loose wispy tracts and Paratangential layer in the ectosome; *Hymeniacidon caruncula* sensu Stephens (1915), D. in situ; E. Photomicrograph of spicule compliment, styles; *Hymeniacidon styliferus* Stephens, 1915, syntype, F. in situ; G. Photomicrograph of spicule compliment, styles.

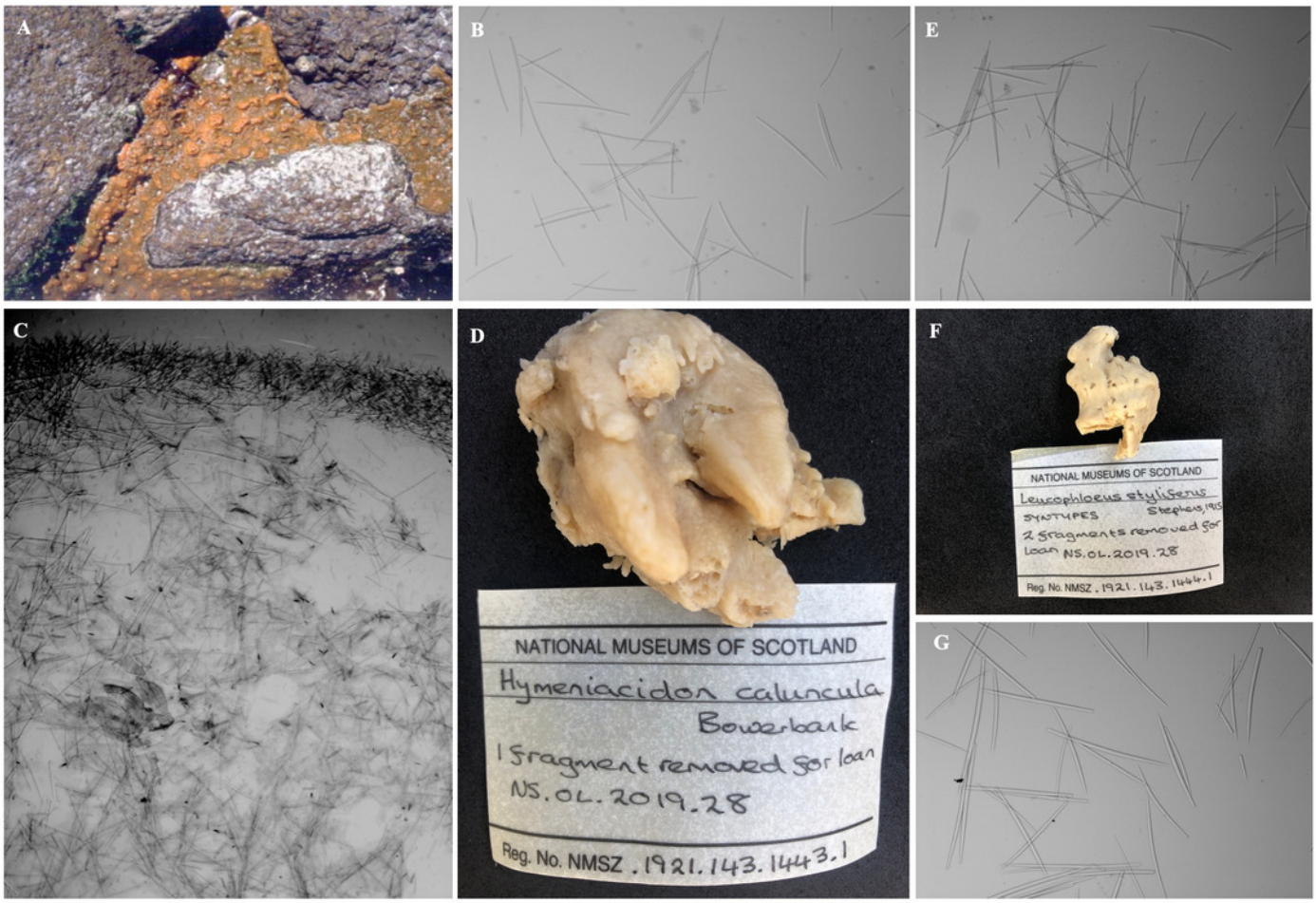

Figure 8

Megasclere spicule lengths for the South African and Global species of *Hymeniacidon perlevis*.

Megasclere spicule lengths for the South African and Global species of *Hymeniacidon perlevis*. Each point represents the max and min spicule length for a specimen.

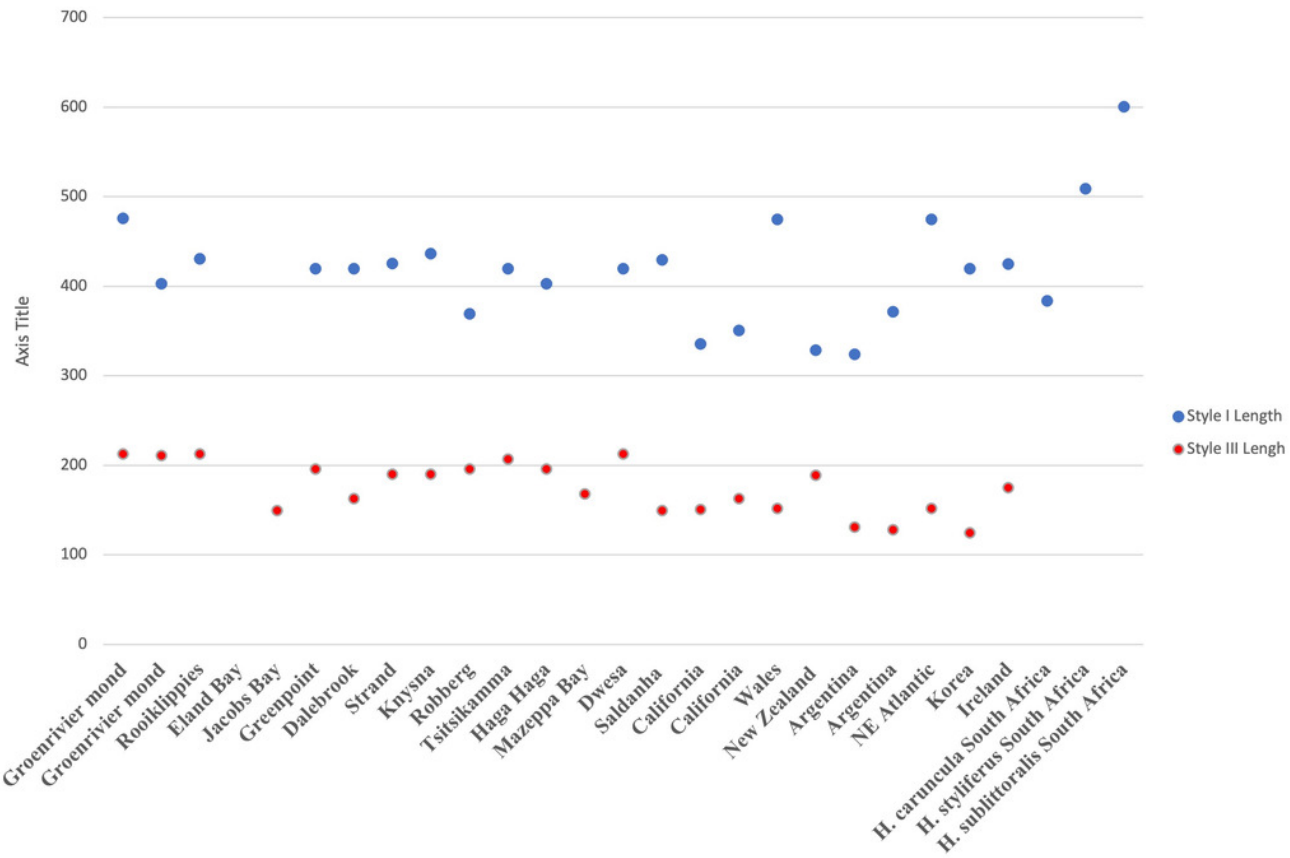

Table 1(on next page)

Comparative micrometric data of spicules for voucher specimens of *H. perlevis* from South Africa. Micrometric values in µm

Comparative micrometric data of spicules for voucher specimens of *H. perlevis* from South Africa. Micrometric values in µm.

1 **TABLE 1. Comparative micrometric data of spicules for voucher specimens of *H. perlevis* from South Africa.**

2 **Micrometric values in µm**

Species	Specimen	Location	Large style	Medium style	Small style
Hymeniacidon perlevis	TS305	Jacobs Bay	337 × 7 µm		155 × 7 µm
	TS1163	Knysna	381 (336–437) × 5 µm	270 (224–324) × 2.4 µm	168 (146–190) × 2.4 µm
	TS1167	Tsitsikamma	386 (347–420) × 5 µm	274 (246–308) × 2.4 µm	167 (145–207) × 2.4 µm
	TS1189	Robberg	359 (336–370) × 5 µm	285 (235–308) × 4.8 µm	157 (140–196) × 4.8 µm
	TS2736	Greenpoint	375 (336–420) × 11.2 µm	290 (246–314) × 11.2 µm	162 (140–224) × 5.6 µm
	TS2736	Dalebrook	374 (336–420) × 11.2 µm	290 (252–308) × 11.2 µm	163 (140–196) × 5.6 µm
	TS2743	Strand	390 (358–427) × 11.2 µm	294 (280–308) × 11.2 µm	187 (168–190) × 5.6 µm
	TS2765	Mazeppa		307 (280–336) × 11.2 µm	144 (112–168) × 5.6 µm
	TS2935	Groenrivier Mund	416 (364–476) × 11.2 µm	274 (224–336) × 5.6 µm	194 (179–213) × 5.6 µm
	TS2963	Rooiklippies	493 (364–431) × 11.2 µm	288 (246–336) × 11.2 µm	198 (190–213) × 5.6 µm
	TS3359	Dwesa	378 (358–420) × 5.6 µm	321 (302–336) × 5.6 µm	187 (157–213) × 5.6 µm
	TS4860	Haga Haga	375 (336–403) × 5.6 µm	321 (302–336) × 5.6	187 (157–213) × 5.6 µm

				μm	

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