nature portfolio

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Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our Editorial Policies and the Editorial Policy Checklist.

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed	
☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒	A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒	The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☒	☐	A description of all covariates tested
☐	☒	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒	A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒	For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☒	☐	Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	Data was produced by us in the laboratory
Data analysis	BRAKER 2.1.6 Augustus 3.5.0 StrinTie 2.2.1 GenomeThreader 1.7.1 Mikado 2.3.4 STAR 2.7.10b TransDecoder 5.7.1 CD-Hit 4.8.1 Portcullis 1.1.2 gffread 0.11.7 Repeatmodeller FindZX mosdepth 0.3.2 samtools 1.14 vcftools 0.1.16 bcftools 1.12 SexFindR

kmersGWAS
PLINK
ABySS
D-GENIES
iDIG
ggplot2
RADsex 1.2
FASTQC
Stacks 2.61
blast
HISAT2
SortMeRNA 4.3.6
BowTie
RSEM 1.2.21
edgeR
Blast2GOPRO
ShinyGO
rMATS 4.3.0
OrthoFinder 2.5.4
macrosyntR
HMMER 3.4
MAFFT 7.525
BMGE 1.12
FastTree 2.1.11
IQ-TREE 2.3.6
OrthoVenn 3
GenEra 1.4.2
DIAMOND 2.1.9

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

Raw genomic and transcriptomic sequences are deposited in NCBI Bioprojects PRJNA1336057, PRJNA1274490.

Research involving human participants, their data, or biological material

Policy information about studies with human participants or human data. See also policy information about sex, gender (identity/presentation), and sexual orientation and race, ethnicity and racism.

Reporting on sex and gender	N/A
Reporting on race, ethnicity, or other socially relevant groupings	N/A
Population characteristics	N/A
Recruitment	N/A
Ethics oversight	N/A

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☐ Life sciences ☐ Behavioural & social sciences ☒ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Ecological, evolutionary & environmental sciences study design

All studies must disclose on these points even when the disclosure is negative.

Study description	To investigate sex determination across Porifera, we conducted a phylogeny-wide genome sampling of six sponge gonochoristic species, and two more gonochoristic sponges for which a chromosome-level genome was not available and transcriptomic data was used instead. But before processing the tissue samples for sequencing, we determined the sex of each individual sponge by surveying the tissue for gametes, finding female and male gametes always in different individuals, pointing to different sex ratios in the populations surveyed, with female-skewed ratios in Chondrosia reniformis, Geodia hentscheli, Petrosia ficiformis and Halichondria panicea, and male-skewed ratios in the rest of species.
Research sample	We sampled 8 sponges with separate sexes (Oscarella lobularis, Chondrosia reniformis, Geodia hentscheli, Geodia barretti, Petrosia ficiformis, Axinella damicornis, Phakellia ventilabrum and Halichondria panicea). We collected 136 samples for identification of sex, and 50 females and 39 males, with at least 3 replicates per species for RADseq, but from 1 to 6 for whole genome sequencing, and from 1 to 10 for RNAseq. For whole genome sequencing, having a reference chromosomal genome for comparison, one individual is enough. For RNAseq, although 1 individual indicates low replication, this only happened in Geodia barretti, a deep-sea species that is hard to collect, especially during the reproductive period, since only 25% of the population is engaged in reproduction.
Sampling strategy	Sampling was performed using SCUBA diving for those species whose depth range is less than 20 m (Oscarella lobularis, Chondrosia reniformis, Geodia hentscheli, Geodia barretti, Petrosia ficiformis, Axinella damicornis, and Halichondria panicea), and using trawling and ROV collectors for deep-sea species (Geodia barretti, Geodia hentscheli, and Phakellia ventilabrum). We collected all specimens living in the surveyed areas for identification of sex but only those producing gametes could be used for subsequent purposes of sequencing.
Data collection	Samples were collected by Ana Riesgo, Vasiliki Koutsouveli, Aida Verdes, Patricia Álvarez-Campos, Ute Henschel, Sergi Taboada, and María Conejero during sampling campaigns with SCUBA diving in 2021 in Blanes bay (Spain), and in 2022 in Naples (Italy) and Kiel Bight (Germany) for shallow-water sponges. For deep–sea sponges, campaigns to collect material were undertaken in Korsfjord, Nowrway/Langenuen and Korsenfjord, Sweden in 2016, and W Rosemary Bank Seamount in 2018 with trawl nets and ROVs.
Timing and spatial scale	Samples were collected by Ana Riesgo, Vasiliki Koutsouveli, Aida Verdes, Patricia Álvarez-Campos, Ute Henschel, Sergi Taboada, and María Conejero during sampling campaigns with SCUBA diving in 2021 in Blanes bay (Spain), and in 2022 in Naples (Italy) and Kiel Bight (Germany) for shallow-water sponges. For deep–sea sponges, campaigns to collect material were undertaken in Korsfjord, Nowrway/Langenuen and Korsenfjord, Sweden in 2016, and W Rosemary Bank Seamount in 2018 with trawl nets and ROVs.
Data exclusions	We did not exclude any data.
Reproducibility	No experiments were performed
Randomization	For identification of sex-differences in coverage, heterozygosity, and presence of sex-specific loci, analyses were performed with the sex assigned based on histological analyses, and then randomised.
Blinding	Blinding was not relevant since no experimentation was performed

Did the study involve field work? [x] Yes [ ] No

Field work, collection and transport

Field conditions	Fieldwork was undertaken during the summer (July and August) in 2021 in Blanes bay (Spain), and in 2022 in Naples (Italy), when seawater temperature was at the highest in the Mediterranean (aroun 26ºC). In Kiel Bight, samples of Halichondria panicea were taken from February 2022 to September 2022 covering the range of temperatures in the field. For samples of deep sea sponges sampling was performed during September in Korsfjord, Nowrway/Langenuen and Korsenfjord, Sweden in 2016, and in August in W Rosemary Bank Seamount and Vesterisbanken in 2018 with trawl nets and ROVs, when the reproductive period of sponges is known to occur.
Location	For shallow water sponges, sampling in Blanes Bay was done in Santa Anna point (41.6734, 2.8026), sampling in Italy was done in Ischia (40.6965, 13.8833), and sampling in Germany was done in Kiel Bight (54.452695, 10.198803), around 5–10 m depth. Sampling in Greenland was done in a submarine seamount (Vesterisbanken, 73.5195, 9.113111) at 580 m depth, in Norway in Korsjfjord (59.9813, 5.3738) at 80 m and in Sweden in Korsenfjord (59.8728, 5.54989) also at 80 m. Finally, sampling in the Rosemary Bank was done on the seamount (59.2, -10.25) at 1034 m depth.
Access & import/export	All sampling was performed according to European laws, and permits were not necessary since all sampling was done outside MPAs.
Disturbance	Disturbance of the sea bottom only occurred during trawling in Korsfjord and Korsenfjord, and we minimised it by reducing the trawling time to the mininum necessary for collecting enough samples of sponges.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☐	☐ Antibodies
☐	☐ Eukaryotic cell lines
☐	☐ Palaeontology and archaeology
☐	☒ Animals and other organisms
☐	☐ Clinical data
☐	☐ Dual use research of concern
☐	☐ Plants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used	n/a
Validation	n/a

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	n/a
Authentication	n/a
Mycoplasma contamination	n/a
Commonly misidentified lines (See ICLAC register)	n/a

Palaeontology and Archaeology

Specimen provenance	n/a
Specimen deposition	n/a
Dating methods	n/a

☐ Tick this box to confirm that the raw and calibrated dates are available in the paper or in Supplementary Information.

Ethics oversight	n/a

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Animals and other research organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research, and Sex and Gender in Research

Laboratory animals	*For laboratory animals, report species, strain and age OR state that the study did not involve laboratory animals.*
Wild animals	All specimens of the species Oscarella lobularis, Chondrosia reniformis, Geodia hentscheli, Geodia barretti, Petrosia ficiformis, Axinella damicornis, Phakellia ventilabrum and Halichondria panicea were collected in their natural habitats. For Oscarella lobularis, Chondrosia reniformis, Petrosia ficiformis, Axinella damicornis, and Halichondria panicea, only a small fraction of about 2 cm3 was collected and the individual was left in the field for recovery. For Geodia barretti, Geodia hentscheli and Phakellia ventilabrum, entire individuals were collected. All samples (whether fragments or entire specimens) were immediately preserved in formaline, RNAlater or absolute ethanol for further processing.
Reporting on sex	Sex was a fundamental aspect of the study and was identified after collection by inspection of histological preparations of the tissue when they presented gametes. It was recorded and all sequencing was performed on female and male specimens.
Field-collected samples	All samples were collected in the field, immediately preserved after collection (on board or in the field after diving) and stored at appropriate temperatures (room temperature for samples in formalin, -20ºC for samples in absolute ethanol, and -80ºC for samples in RNAlater.

Ethics oversight	No ethical approval is necessary for collection of sponges (invertebrates without nervous system).

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Clinical data

Policy information about clinical studies

All manuscripts should comply with the ICMJE guidelines for publication of clinical research and a completed CONSORT checklist must be included with all submissions.

Clinical trial registration	*Provide the trial registration number from ClinicalTrials.gov or an equivalent agency.*
Study protocol	*Note where the full trial protocol can be accessed OR if not available, explain why.*
Data collection	*Describe the settings and locales of data collection, noting the time periods of recruitment and data collection.*
Outcomes	*Describe how you pre-defined primary and secondary outcome measures and how you assessed these measures.*

Dual use research of concern

Policy information about dual use research of concern

Hazards

Could the accidental, deliberate or reckless misuse of agents or technologies generated in the work, or the application of information presented in the manuscript, pose a threat to:

No	Yes	
☒	☐	Public health
☒	☐	National security
☒	☐	Crops and/or livestock
☒	☐	Ecosystems
☒	☐	Any other significant area

Experiments of concern

Does the work involve any of these experiments of concern:

No	Yes	
☒	☐	Demonstrate how to render a vaccine ineffective
☒	☐	Confer resistance to therapeutically useful antibiotics or antiviral agents
☒	☐	Enhance the virulence of a pathogen or render a nonpathogen virulent
☒	☐	Increase transmissibility of a pathogen
☒	☐	Alter the host range of a pathogen
☒	☐	Enable evasion of diagnostic/detection modalities
☒	☐	Enable the weaponization of a biological agent or toxin
☒	☐	Any other potentially harmful combination of experiments and agents

Plants

Seed stocks	*Report on the source of all seed stocks or other plant material used. If applicable, state the seed stock centre and catalogue number. If plant specimens were collected from the field, describe the collection location, date and sampling procedures.*
Novel plant genotypes	*Describe the methods by which all novel plant genotypes were produced. This includes those generated by transgenic approaches, gene editing, chemical/radiation-based mutagenesis and hybridization. For transgenic lines, describe the transformation method, the number of independent lines analyzed and the generation upon which experiments were performed. For gene-edited lines, describe the editor used, the endogenous sequence targeted for editing, the targeting guide RNA sequence (if applicable) and how the editor was applied.*
Authentication	*Describe any authentication procedures for each seed stock used or novel genotype generated. Describe any experiments used to assess the effect of a mutation and, where applicable, how potential secondary effects (e.g. second site T-DNA insertions, mosiacism, off-target gene editing) were examined.*