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	No software was used for data collection.
Data analysis	The authors declare that all code is available in the supplementary information files. We transformed the maximum likelihood topology into an ultrametric tree and conducted ancestral state reconstructions in R (version 4.3.0).

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

The authors declare that all data supporting the findings of this study are available within the paper and its supplementary information files.

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	We compiled a comprehensive list of all known fluorescent ray-finned fishes and conducted ancestral state reconstructions on a time calibrated actinopterygian phylogeny of the absence and presence of biofluorescence.
Research sample	Biofluorescent presence was determined through a literature search of peer-reviewed descriptions of biofluorescence in addition to our own novel observations.
Sampling strategy	Live and frozen specimens used for examination and imaging were collected in the Solomons Islands in 2012, 2013, and 2019, Greenland in 2019, the Bahamas (Exumas) in 2011 and 2012, the Cayman Islands in 2016, Southern California (San Diego) in 2015 and 2016, near-shore locations in Washington in 2022, Florida in 2022, and obtained through the aquarium trade. Additional fluorescence imaging was conducted at the Mystic Aquarium, Mystic, CT in 2011 and 2012, and at the Birch Aquarium, Scripps Institution of Oceanography, La Jolla, CA in 2015 and 2016. Sample sizes (n=1-2) of newly described biofluorescent species can be found in the supplementary files (S2).
Data collection	All authors contributed to data collection. For live-specimen examination, we placed individual fish in a narrow photographic tank and gently held them flat against the thin glass front. We imaged fluorescent emissions in a dark room using a Nikon D800 or D4 DSLR camera outfitted with a Nikon 60 or 105 mm macro lens, or a Sony A7SII camera outfitted with a Sony 90 mm macro lens. We covered the flashes (Nikon SB910) with blue interference bandpass excitation filters (Omega Optical, Inc., Brattleboro, VT; Semrock, Inc., Rochester, NY) and attached longpass (LP) emission filters (Semrock, Inc.) to the front of the camera lens to record any emitted fluorescence.
Timing and spatial scale	The authors have recorded the presence of biofluorescence in actinopterygians from 2011-present.
Data exclusions	No data was excluded from this study.
Reproducibility	All attempts to repeat the experiment were successful.
Randomization	This is not relevant to this study as the presence of biofluorescence is based on spectrophotometer measurements of shifts in wavelengths.
Blinding	This is not relevant to this study as the presence of biofluorescence is based on spectrophotometer measurements of shifts in wavelengths.

Did the study involve field work? ☒ Yes ☐ No

Field work, collection and transport

Field conditions	Field conditions varied greatly, but did not affect the results of this study.

Location	Live and frozen specimens used for examination and imaging were collected in the Solomons Islands in 2012, 2013, and 2019, Greenland in 2019, the Bahamas (Exumas) in 2011 and 2012, the Cayman Islands in 2016, Southern California (San Diego) in 2015 and 2016, near-shore locations in Washington in 2022, Florida in 2022, and obtained through the aquarium trade. Additional fluorescence imaging was conducted at the Mystic Aquarium, Mystic, CT in 2011 and 2012, and at the Birch Aquarium, Scripps Institution of Oceanography, La Jolla, CA in 2015 and 2016.
Access & import/export	Research, collecting, and export permits were obtained from the government of the Bahamas, from the Ministry of Fisheries and Ministry of Environment, Honiara, Solomon Islands, and from the Department of Environment, Cayman Islands Government.
Disturbance	N/A

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

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	N/A
Wild animals	Various species of ray-finned fishes were observed and collected (see supplementary file S2). Fishes were collected via SCUBA through the application of rotenone and quinaldine to a targeted variety of shallow to deep (mesophotic) habitats in each sampling location where collecting was permitted.
Reporting on sex	N/A
Field-collected samples	No live fish were collected and housed during this study.
Ethics oversight	This study was carried out in strict accordance with the recommendations in the Guidelines for the Use of Fishes in Research of the American Fisheries Society and the American Museum of Natural History's Institutional Animal Care and Use Committee (IACUC).

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

Plants

Seed stocks	N/A
Novel plant genotypes	N/A
Authentication	N/A