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Corresponding author(s):	Jonathan Jung and Alfredo Martinez-Garcia

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Reporting Summary

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

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n/a	Confirmed
	$oxed{oxed}$ 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.
\times	A description of all covariates tested
\times	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. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted Give <i>P</i> values as exact values whenever suitable.
\times	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
\times	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
\times	Estimates of effect sizes (e.g. Cohen's <i>d</i> , Pearson's <i>r</i>), indicating how they were calculated

Our web collection on <u>statistics for biologists</u> contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection

All analyses were conducted using Python3 on a Jupyter Notebook® (version 5.7.4). Data were imported using the Pandas library and plotted with Seaborn or Matplotlib libraries.

The nitrogen isotopes were determined by a purpose-built inlet system coupled to a Thermo MAT253 Plus stable isotope ratio mass spectrometer (software: Isodat version 3.0). Oxygen and carbon isotopes were were measured with an isotope ratio mass spectrometer (IRMS) (Delta V Advantage, Thermo Scientific, Bremen, Germany) which is connected to a GasBench II unit (Thermo Scientific) (software: Isodat version 3.0).

Data analysis

No software was used for data analysis and the codes used for figures and data analyses are available on GitHub (https://github.com/marinejon/Coral-Photosymbiosis-on-Mid-Devonian-Reefs.git)

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

All data are available in the Supplementary Tables 2 - 5 and upon publication data will be stored in Pangaea (https://www.pangaea.de/)

Research involving human participants, their data, or biological material

Policy information about studies with <u>human participants or human data</u>. See also policy information about <u>sex, gender (identity/presentation)</u>, <u>and sexual orientation</u> and <u>race</u>, 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 science
Life selettices	beliavioural & social sciences	Leological, evolutionally & environmental science

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

Pairs of modern symbiont-bearing and symbiont-barren samples were analyzed to define the isotopic offset (carbon, oxygen and nitrogen isotopes) between the two ecological groups. Samples of Paleozoic groups and different morphologies were analyzed for carbon, oxygen and nitrogen isotopes and compared to the modern pairs. Carbon and oxygen isotopes were used because of prior studies and to show that the direct comparison between modern and Paleozoic corals does not yield a comprehensive picture to differentiate between ancient symbiont-bearing and -barren species. Nitrogen isotopes analyses were used as a new way to distinguish between ecological groups of the Paleozoic.

Research sample

Fossilized coral samples of the groups Tabulata and Rugosa from the Paleozoic taken from the Sauerland and Eifel region in Germany as well as Tafilalt in Morocco and Sabkhat Lafayrina in West Sahara. Furthermore, we analyzed modern scleractinian corals from several locations as indicated in Extended Data Figure 2 and Supplementary Table 3 & 5.

Sampling strategy

For the Paleozoic samples, we used specimens that provided sufficient material to compare uncleaned and cleaned material (as indicated in Figure 2 and Extended Data Figure 4). Sample material was drilled with a hand-dremel (0.9 mm drill-bit) and material was only taken from the calcitic phase (as indicated in Extended Data Figure 1). For modern samples, material was drilled from samples that were taken from the same reef location and depth. Coral pieces (~2x2 cm) were cut and crushed in an Achat mortar before being cleaned.

Data collection

Nitrogen Isotope data were analyzed at the Stable Isotope Laboratory of the Max-Planck Institute for Chemistry in Mainz, Germany. Data were mainly analyzed by Jonathan Jung with the supervision of Alfredo-Martinez-Garcia. Carbon and Oxygen Isotopes were analyzed in the inorganic stable isotope laboratory at the Max-Planck-Institute for Chemistry in Mainz, Germany.

Timing and spatial scale

Isotopic analysis were conducted between October 2019 and December 2023. Paleozoic coral samples were taken from two locations in Germany with different diagenetic histories as well as two locations from the opposite basin of the Rheic Ocean (Morocco and West Sahara) and compared to modern coral samples from a range of underlying environmental conditions from which we expected different nitrogen isotope values for symbiont-bearing corals. Our rational was to show that despite different

	underlying environmental conditions (i.e., oligotrophic vs. eutrophic) the difference between symbiont-bearing and symbiont-barren nitrogen isotope values stays the same.		
Data exclusions	No samples were excluded.		
Reproducibility	Samples of the same specimens were analyzed over several batches and reproducibility is given as the standard deviation.		
Randomization	Samples did not need to be grouped since there are no inter-dependencies between species.		
Blinding	Samples were analyzed in a random order but had to be indicated by labels.		
Did the study involve field	l work? X Yes No		
ield work, collect	tion and transport		
Field conditions	At Sauerland, hand pieces were collected off the ground. Collection happened in October 2018, while It was cloudy but dry at 13°C.		
Location	The main material was collected near a cliff at the top of the Binolen section ("C-Layers" after Löw et al., 2022; GPS: 51°22′12″N, 7° 51′27″E) within the Hönne Valley in north-western Sauerland.		
Access & import/export	Samples were taken close to a publicly accessible forest trail where no permits are needed. Pieces were exclusively collected from the ground.		
Disturbance	Only those hand-pieces were collected that could be picked up from the ground.		
Materials & experime /a Involved in the study Antibodies Eukaryotic cell lines Palaeontology and an Animals and other o Clinical data Dual use research of Plants	n/a Involved in the study ChIP-seq Flow cytometry rchaeology MRI-based neuroimaging rganisms		
alaeontology and	d Archaeology		
Specimen provenance	Sampling permits for specimens from the the Senckenberg Research Institute and Natural History Museum Frankfurt, Germany were issued. No permits were needed otherwise.		
Specimen deposition	Thin sections for species identification from the initial Hagen Balve Reef at Binolen will be stored in the Geomuseum of the Westfälische Wilhelms University in Münster (GMM) under the inventory numbers GMM B2C.59-1 to GMM B2C.59-9. Sample material from the Eifel and the modern samples are stored at the Senckenberg Research Institute and Natural History Museum Frankfurt, Germany. All sample aliquots are stored at the Max-Planck Institute for Chemistry in Mainz.		
Dating methods	No new dates were obtained.		
Tick this box to confirm	n that the raw and calibrated dates are available in the paper or in Supplementary Information.		
Ethics oversight	No ethical approval was necessary given that no endangered species were analyzed.		

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

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

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.