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Title: Pollinators of the sea: a discovery of animal mediated fertilization in seaweed

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Abstract: The long-held belief that animal-mediated pollination is absent in the sea has recently been contradicted in seagrasses, motivating investigations of other marine phyla. This is particularly relevant in red algae where female gametes are not liberated and male gametes not flagellated. Using experiments with the isopod *Idotea balthica* and the red alga *Gracilaria gracilis*, we demonstrate that biotic interactions dramatically increase the fertilization success of the alga

by animal transport of spermatia on their body. This discovery suggests that animal-mediated fertilization could have evolved independently in terrestrial and marine environments and raises the possibility of its emergence in the sea before plants moved ashore.

One-Sentence Summary:

The mesograzer isopod *Idotea balthica* facilitates the transfer of spermatia in red alga *Gracilaria gracilis*.

Main text:

Animal-mediated pollination in plants is believed to have originated more than 140 million years ago (Mya) (1, though this date still sparks debate, see 2). Insect pollination is the most common form of fertilization in flowering plants (3) and is usually the unintended consequence of an animal's feeding activity. While extensively studied on land, a demonstration of animal pollination in the marine environment was made in 2012 when foraging marine invertebrates were shown to carry and transfer pollen grains from male to female flowers in the seagrass *Thalassia testudinum* K.D.Koenig (4, 5). This finding contradicted the long-held belief that animal-mediated pollination is absent in the sea (6) and begged the question of its presence in other marine life, particularly in seaweeds.

Here we investigated whether animal-mediated dissemination of male gametes occurs in seaweed. This question is particularly relevant in Florideophyceae since male gametes are not flagellated or motile, similar to pollen grains, and fertilization takes place on a remote female organ. The sperm (spermatia) cannot swim to attach to the trichogyne (the female receptive structure) and are typically thought to be dispersed only passively through water flows (7).

the marine isopod *Idotea balthica* (Pallas, 1772) is commonly found firmly gripped to the red alga (Fig. 1A, Movie S1) *Gracilaria gracilis* (Stackhouse) M. Steentoft, L.M. Irvine & W.F. Farnham. Using two sets of experiments, we tested whether *I. balthica* facilitates male gamete dispersal and fertilization in this red alga species, either indirectly, via the creation of water turbulence when swimming, or directly, by carrying spermatia on their bodies. Mating in *G. gracilis* occurs when spermatia released into the environment (Fig. 1B) encounter a trichogyne on the female thallus, where fertilization takes place. After fertilization, the zygote develops on the female alga into a complex structure visible to the naked eye called a cystocarp (Fig. S1). We, therefore, used the number of cystocarps per centimeter of thallus as a proxy of fertilization success.

In the first set of experiments, virgin reproductive-female thalli were placed in calm seawater aquaria fifteen centimeters apart from reproductive-male thalli, either in the presence or absence of idotea isopods (Fig. 2A, 2B, respectively). Fertilization success was about 20 times higher in the presence of idoteas than without (Fig. 2C, Wilcoxon rank-test, $n=5$ replicates, $p=0.031$). In the second set of experiments, virgin females were placed in aquaria with or without idoteas that had been pre-incubated with reproductive-male thalli (Fig. 2D, 2E, respectively). To control for the possibility that sperm contamination by the operator during animal transfer led to fertilization events, a mock transfer involving plunging the instruments used to handle idoteas was performed at the beginning of experiment 2E. Only two cystocarps were detected –and in only one replicate– after the mock transfer (i.e., an average 0.002 cystocarp/cm, table S1), indicating that such sperm contamination by the operator was negligible. Fertilization success rate reached 0.11 cystocarp/cm of thallus in the presence of incubated idoteas (Fig. 2F, Wilcoxon rank-test, $n=5$ replicates, $p=0.028$), confirming direct gamete transport by isopods. Idoteas were imaged utilizing confocal laser scanning microscopy to demonstrate that they were indeed carrying spermatia. The presence

of spermatia was revealed by staining (Fig. 1C). The cuticles of the animals were studded with spermatia (Fig. 1D, Fig. S2, Movie S2), mainly adhering to the setae of pereopods (Fig. 1E, Movie S2) and on the margin segments (Fig. 1F, Movie S2) due to the sticky nature of the sperm mucilage (Fig. S3).

Our results demonstrate for the first time that biotic interactions dramatically increase the probability of fertilization in a seaweed. The observation of mucilage-embedded spermatia attached to idotea body parts after visiting male gametophytes suggests that idotea may serve as “pollinators” in *G. gracilis* upon subsequent visits to female individuals (Movie S1). If we draw a parallel with the Cox-Knox Postulates defining criteria for determining pollinators of flowering plants (8), our results are sufficient to prove that idoteas are efficient gamete-vectors.

This discovery is particularly relevant in Rhodophyta as male gametes are not flagellated, have a short lifespan (9), and more than 75% of species are dioicous (having male and female structures on different individuals), which means that fertility success is distance-dependent and limited by mate density (10). Syngamy in red algae is expected to be relatively rare compared to marine organisms possessing external fertilization, the synchronous release of motile gametes, and chemotaxis (e.g., invertebrates and brown seaweeds) unless compensatory mechanisms increase sperm production, sperm transfer or zygote survival. Searles (1980) hypothesized that the evolution of a life history in which the female nurtures and protects the zygote is probably a response to low rates of fertilization in both plants and red algae (i.e., the cystocarps). Despite debate concerning the compensatory allocation of resources for zygote survival in red algae, the possibility of a biotic interaction facilitating fertilization success in this group had not been previously explored. In particular, animal-mediated transport of spermatia in red algae could dramatically increase fertilization success in calm rock pools (a condition that was mimicked by

our experimental conditions) at low tide, when most fertilizations have been detected (11). However, the relative importance of animal-mediated transport versus water movements in fertilization success was not tested. Intertidal seaweed could combine the advantages of both water and animal dispersal of male gametes when alternating between low and high tide conditions.

While *I. balthica* grazes on other seaweed, it does not feed directly on *Gracilaria* but rather eliminates epiphytes at the surface of the thallus while protecting itself from predators (12-14). We suggest that the relationship could be mutually beneficial. For *I. balthica*, the seaweed provides shelter and epiphytic diatoms found adhering to the surface of the thallus, and whose frustules (or thecae) are found in the feces of idoteas (Fig. S4), appear to be an important food source. In return, *G. gracilis* benefits through increased growth rate due to reduced fouling (15) and improved reproductive success (present study). The benefit of animal-mediated “pollination” may be particularly important in species like *G. gracilis* with funnel-shaped trichogynes that do not protrude from the surface of the thallus (16), making fertilization more difficult compared to other Rhodophyta such as *Bostrychia moritziana* (17), which have elongated trichogyne (about 100 microns long). As in flowering plants where ambophily (i.e., adaptation to both wind and animal pollination) appears more important than previously reported (18), our results demonstrate that in addition to the well-established role of water movement (7, 9, 10), animal-mediated fertilization is also present in the sea. This observation opens up new unexplored avenues of research aimed at understanding the ecology of these interactions.

Animal pollination is generally thought to have originated during the Mesozoic for gymnosperms and angiosperms and was believed absent in mosses and ferns, in which fertilization is restricted to water (19-21). However, transport of male gametes by animals has been demonstrated recently in mosses, antedating animal-mediated pollination at the early phase of land colonization (circa

450 Mya, 21). The discovery of animal-mediated fertilization in Florideophyceae, whose origin was estimated between 817 and 1049 million years (22), suggests that it could have arisen with the diversification of the metazoan some 650 Mya (23), even before plants moved ashore. However, we cannot rule out that different animal-mediated fertilization mechanisms evolved independently and repeatedly in terrestrial and marine environments.

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Author contributions:

EL, CD, MLG and MV designed the experiment; EL, CD, AF and MV performed the field sampling; EL, JC and AF conducted the laboratory work; EL, AF and MV performed the statistical analyses; SC designed and conducted the CLSM experiment, analyzed the imaging data and prepared the corresponding figures; EL, SC, CD, MLG and MV wrote and revised the manuscript, with further edits from all authors; MLG and MV obtained the funding; MV designed and supervised the project with CD and MLG.

Data availability: All data used in the analyses are available in the paper or given in the supplementary material

Supplementary Materials

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Materials and Methods

Figs. S1 to S4

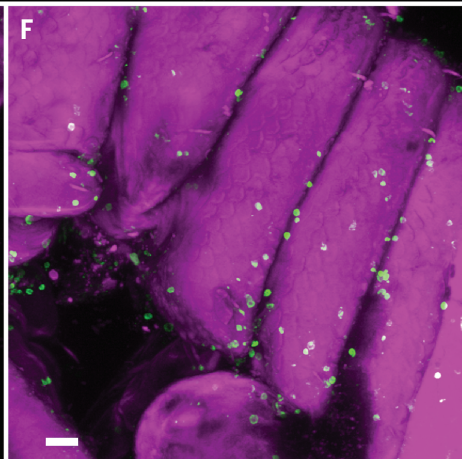
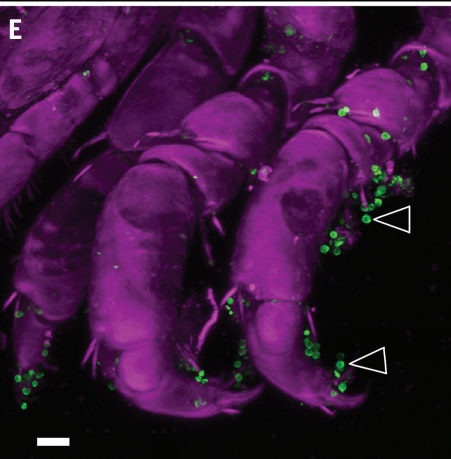
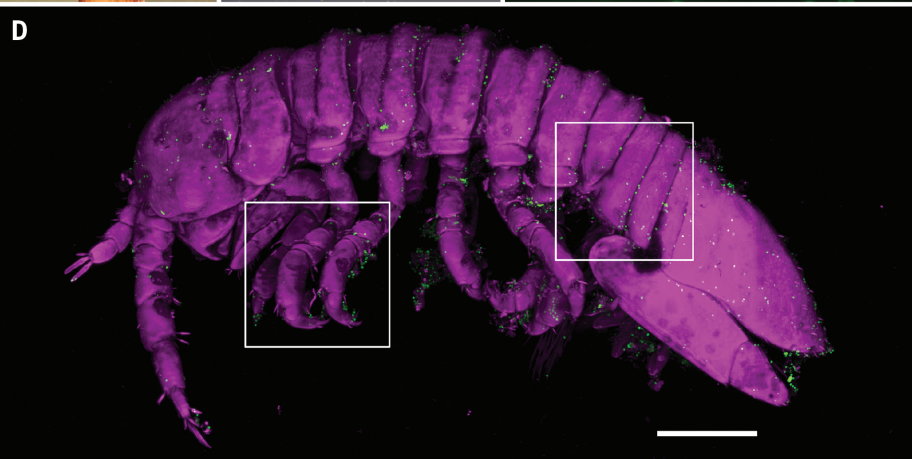
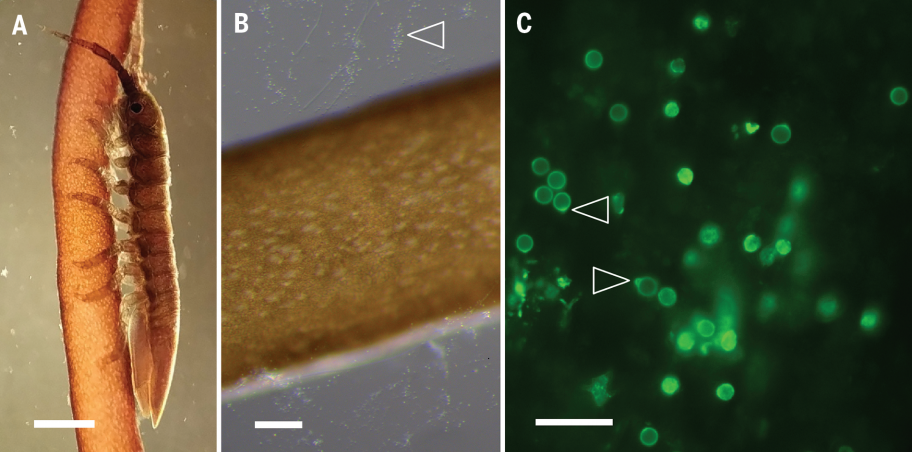
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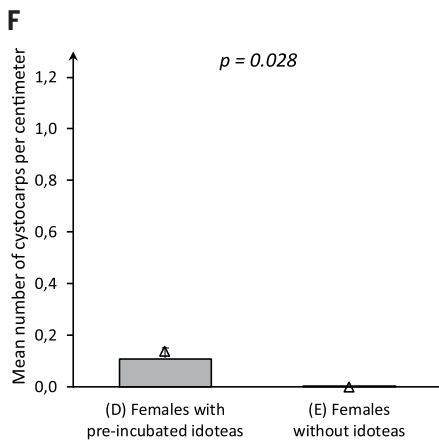
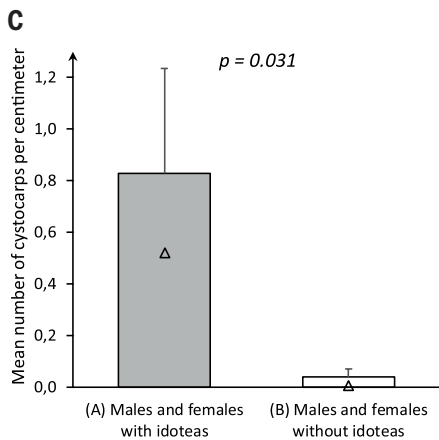
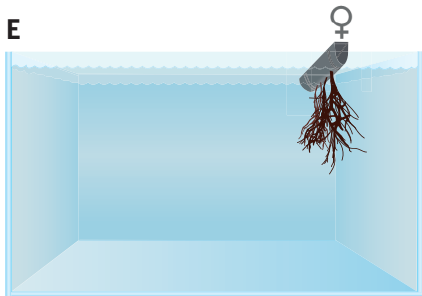
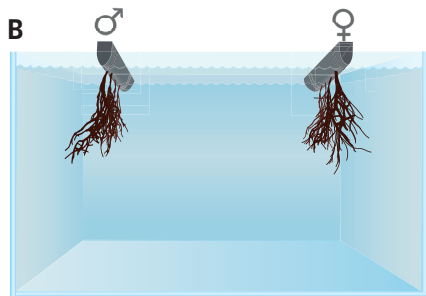
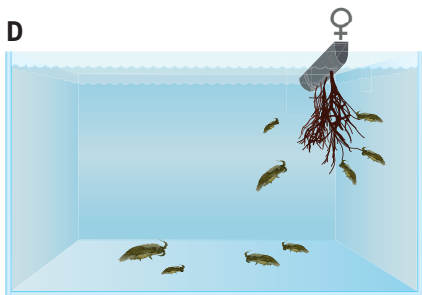
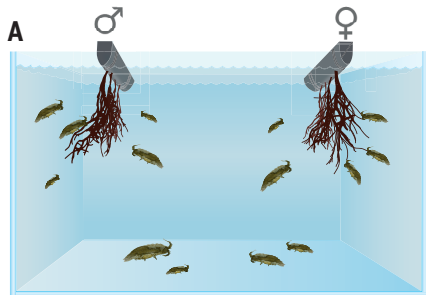
Movies S1 to S2

Fig. 1. The idoteas carry spermatia of *Gracilaria gracilis* on their bodies

(A) *Idotea balthica* firmly clinging to the reproductive-male thallus of *G. gracilis* (oblique illumination, macroscopy, see also Movie S1). The light spots on the thallus correspond to the male conceptacles. (B) Release of spermatia (arrowhead) at the surface of the reproductive-male thallus (oblique illumination, macroscopy). (C) Spherical spermatia about 4.5 μ m in diameter (arrowheads) with their noticeable bump (epifluorescence microscopy, staining with the lectine Concanavalin A conjugated to the fluorochrome (AlexaFluor488). ConA-AF488 binds to specific carbohydrates (24) on the surface of the cell and mucilage). (D) 3D reconstruction from confocal laser scanning microscopy (CSLM) of a young specimen of *I. balthica* collected on a reproductive-male gametophyte of *G. gracilis* (tegument of the isopod was fixed and stained with calcofluor white (CFW) and the spermatia with ConA-AF488 (see also Fig. S2 and S3 and Movie S2). (E) Close-up of (D)-insets (right): location of spermatia on the setae of pereopods (arrowheads). (F) Close-up of (D)-insets (left): location of spermatia on the margin of segments. Scale bars are 1 mm in panel (A), 200 μ m in panels (B) and (D) and 20 μ m in panels (C), (E) and (F).

Fig. 2. Impact of idoteas on fertilization success. (A) Aquarium containing male and female *Gracilaria gracilis* with twenty *Idotea balthica* (Movie S1) and (B) Male and female *G. gracilis* without *I. balthica*. (C) Comparison of the mean number of cystocarps as a proxy of fertilization success between experiments A and B. (D) Females of *G. gracilis* with twenty *I. balthica* pre-incubated with male gametophytes (E) Negative control with females of *G. gracilis* and mock transfer of idoteas. (F) Comparison of the mean number of cystocarps between experiments D and E. Values represented on the graph are the means and the standard error for the five replicates. Medians are indicated by a triangle symbol. P-values are indicated on the graph (unilateral rank test of Wilcoxon for paired samples). Aquarium illustrations by E. Mardones.







Supplementary Materials for

Pollinators of the sea: a discovery of animal mediated fertilization in seaweed

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Materials and Methods
Figs. S1 to S4
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Caption for movie S1

Other Supplementary Materials for this manuscript include the following:

Movie S1
Movie S2

Materials and Methods

Seaweed material:

Gracilaria gracilis has a haplo-diplophasic life cycle with isomorphic male and female (haploid) gametophytes and tetrasporophytes (diploid). Tetrasporophytes produce haploid spores from meiotic divisions that develop into dioecious male and female gametophytes. Spermata (non-flagellated male gametes) are released into the environment while female gametes remain on the female. After fertilization, the zygote is retained on the female and develops into diploid sporogenous tissue protected and nurtured by the parental female thallus. This complex structure visible to the naked eye (about 1 mm in diameter) on the surface of the female thallus is called the cystocarp (Fig. S1). Thousands of diploid spores are released by a cystocarp into the environment to give rise to new diploid tetrasporophyte individuals. Fertilization success is easily quantified by direct counts of cystocarps number per unit of female length.

Both male and female gametophytes of *G. gracilis* were collected from the Cap Gris-Nez (northern France, 50°53'N, 1°35'E). To obtain unfertilized virgin thalli, females were grown for six months at room temperature in 2L flasks of enriched seawater (PES 1mL/L; Provasoli, 1957), under a photoperiod of 12:12, and a light intensity of 45 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$. Reproductive males were harvested one week prior to the experiments and maintained in culture under a 16:8 photoperiod, with a light intensity of 15 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$, and a controlled temperature of 16°C. To confirm spermata production, males were incubated in 500mL of seawater for 30 minutes, and Pluronic™ F-68 (Gibco™, ref:24040032) was added to a 50 mL subsample to maintain cell integrity and reduce the attachment of the cell to the container surface. The preparation was filtered at 0.4 μm . The filter was stained with 50 μL of Concanavalin A (conjugated with Alexa Fluor™ 488, Invitrogen™, Fisher Scientific) at 0.1 mg/mL, left to stand for 20 minutes and then rinsed with 0.2 μm filtered seawater. Gamete presence was confirmed by observation under a LEICA DMI8 fluorescence microscope, objective HC PL FLUOTAR 63x/1.30 OIL and a filter cube for AlexaFluor 488 (Ex480/40 nm BS 505 nm Em 527/30 nm), CCD Camera DFC3000G (Leica).

Animal material:

Idotea balthica were collected on *G. gracilis* thalli from Locquirec (Brittany, France, 48°69'N, 3°66'O). The animals were kept in 10L oxygenated aquaria containing *Ulva lactuca* and *Palmaria palmata* for grazing and *G. gracilis* as shelter.

Verification of species taxonomic status:

Species determination was first done using morphological characters and then verified using the mitochondrial gene COI (cytochrome oxidase subunit 1) for the isopod *I. balthica* and the chloroplast gene *rbcL* (ribulose biphosphate carboxylase large subunit) for the seaweed *G. gracilis*. Sequences were obtained for four *I. balthica* and four *G. gracilis* (two males and two females used in the experiments) following published protocols (25, 26, respectively). Sequences were deposited in Genbank under accession numbers ON239274 to ON239277 for *I. balthica* and ON328328 to ON328331 for *G. gracilis*. In addition, voucher specimens were deposited in the Museum National d'Histoire Naturelle collections (MNHN, Paris, France) under voucher codes MNHN-IU-2021-7259, MNHN-IU-2021-7341, MNHN-IU-2021-6903 and MNHN-IU-2021-

6631 for *I. balthica* and PC0616710 to PC0616713 for *G. gracilis*. The *rbcL* sequences are from the voucher specimens.

Fertilization experiment:

Prior to the experiment, various reproductive fragments of thallus –approximately 10cm long– were taken from eight females and ten males. Groups of male and female thallus fragments were attached to plastic clips (Fig. 2) in order to move the algae easily in and out of the experimental aquaria. All male groups were similar and contained ten thallus fragments, each selected from a different male (the identity of experimental males is given in Table S2). For female groups, three thallus fragments from three randomly selected females were attached to a plastic clip (the identity of experimental females is given in Table S2).

Idotea balthica of varying sizes (1 to 3 centimeters) were isolated 24 hours before the experiments in a 2L oxygenated tank placed in the dark. A synthetic shelter was provided, and *Palmaria palmata* was used as a food source. The animals were then incubated with the clip of 10 selected reproductive males of *G. gracilis* (see above) for two hours. After incubation, animals were transferred to the experimental aquaria using a tea strainer and a brush. The tea strainer containing the animals was rinsed once in filtered seawater to limit the transport of spermatia by remaining seawater before placing 20 *I. balthica* in each experimental aquarium with a clean brush. The experiments were initiated right after the transfer of the isopods to the experimental aquaria.

Four different experimental conditions were established to test the role of *I. balthica* in *Gracilaria gracilis* fertilization (Fig. 2, Table S1). In the first two experimental conditions, males and females of *G. gracilis* were placed about 15 cm from each other in 2L experimental aquaria with or without *I. balthica* for one hour. This distance represents the natural environment where 50% of fertilizations occur between males and females separated by less than 33 cm (10) (Fig. 2A, 2B respectively, Movie S2). The other two experimental conditions correspond to *G. gracilis* females placed alone in 2L experimental aquaria with or without *I. balthica* preincubated with male gametophytes for one hour (Fig. 2D, 2E, respectively). At the beginning of this last experiment, a mock transfer was done to control for possible contamination of tanks by sperm not attached to the isopods. This involved plunging the tea strainer into the experimental aquaria after rinsing with filtered seawater. All experiments were performed in calm seawater to minimize the effect of exogenous water movements on gamete transport and to test only for the effect of isopod activity. All the experiments were replicated five times. After the experiments, each group of female thallus fragments was cultured independently in a 250 mL flask of enriched seawater under constant agitation, under a photoperiod of 12:12 at room temperature. The position of flasks in the culture chamber was randomized every two days. After three weeks, the number of cystocarps (i.e., successful fertilizations) was reported for each thallus fragment (Table S1).

Non-parametric Wilcoxon matched-pairs signed-rank tests (one-sided), using the R-package `wilcox.test` (R Core Team 2021), were used to test for differences in numbers of cystocarps observed per centimeter of female thallus between experiments with and without *idotea*. Tests were performed independently for each pair of experiments (i.e., males and females *G. gracilis* without or with *I. balthica* and females of *G. gracilis* without or with *I. balthica* preincubated with males).

Confocal laser scanning microscopy (CLSM):

Mounting and fluorescent labeling. Idotea and algae were mounted in glass-bottom (#1.5H) microscopy dishes IBIDI (ibidi GmbH, Gräfelfing, Germany) with the appropriate buffer (PBS for fixed specimens, seawater media for live imaging). The labeling/mounting protocols were optimized to reduce specimen manipulation as much as possible. All labeling and washing steps were performed in microcentrifuge tubes with either seawater (live imaging) or PBS (fixed samples).

Observation of male gametes on specimens of *I. balthica*. Isopods were incubated and collected on fertile male gametophytes of *G. gracilis* and then fixed with 4% formaldehyde. The chitinous tegument of the isopod was stained with calcofluor white (CFW, magenta) 0.2 mg/mL final (3h). In contrast, the conjugate Concanavalin A-AlexaFluor488 (ConA-AF488) 0.1 mg/mL final (10 min) was used to label specific carbohydrates (24) of the spermatia of *G. gracilis* and their mucilaginous projection (Fig.1 and Fig. S2).

Observation of live spermatia (Fig. S3). the conjugated ConA-AF488, 0.1 mg/mL final (10 min), was used to label carbohydrates of the spermatia of *G. gracilis* and their mucilaginous projection. Hoechst33342 (Molecular Probes®, H21492) at 5 μ M was used for staining DNA (>30 min, no washing) and nuclei. Chloroplasts were localized from the autofluorescence of chlorophyll (red) and/or phycoerythrin (cyan).

Observation of live epiphyte organisms at the surface of *G. gracilis* (Fig. S4). The staining of the surface of the algae and cellular membranes of epiphytes (green) was achieved with DiOC6(3) (Molecular Probes®, D-273) at 1.5 μ M final (>30 min, no washing). Hoechst33342 (Molecular Probes®, H21492) at 5 μ M was used for staining DNA (>30 min, no washing) and nuclei (Blue). Chloroplasts were localized from the autofluorescence of chlorophyll (red) and/or phycoerythrin (cyan). Epiphytic diatoms at the surface of the thallus were morphologically identified as *Cocconeis scutellum* (27).

Microscopes. CLSM acquisitions were conducted on either a Leica inverted TCS SP8 equipped with a compact light supply unit (solid-state lasers 405/488/552/638 nm), LIAchroics beam splitter, regular PMTs detector, and objectives HC PL APO 40x/1.10 W motCORR CS2, HC PL APO 20x/0.75 IMM CORR CS2), or a Zeiss inverted LSM 780, equipped with the laser beamlines (DPSS 405nm, Argon 488nm, Diode 561nm and 633nm), a 32 channel GaAsP array detector, and objectives C-Apochromat 10x/0.45 W, LD LCI Plan-Apochromat 25x/0,8 Imm Korr DIC and LD C-Apochromat 40x/1,1 W Korr.

Image acquisitions. All multidimensional Z-stacks were acquired with water immersion objectives, averaging (2 to 4 times). The fluorescence channels were acquired sequentially when necessary to avoid crosstalk (e.g., Hoechst or Calcofluor white with AlexaFluor 488). The signal acquisition was oversampled in the optical axis to allow reliable 3D-reconstructions. The pinhole diameter was set to match Airy1 value at the lowest fluorescence wavelength. The spectral settings for excitation and detection of fluorescence emission are: Hoechst, Ex405 / Em 420-470; CFW, Ex405 / Em 420-470; ConA-AF488 Ex488 / Em 500-520; DiOC6(3) Ex 488 / Em 500-520; Phycoerythrin Ex552 or 561 / Em 570-600; Chlorophyll Ex488 and/or638 / Em 680-700.

Image processing. 3D reconstructions from multidimensional Z-stacks, contrast adjustments, and rendering were performed with Imaris (8.0.2, Bitplane). False colors were used to distinguish different channels in the images: Hoechst or CFW, blue; DiOC6(3) or Cona-AF488, green; Phycoerythrin, cyan; Chlorophyll, red. In Figure 1, the contrast of the isopod body (CFW, counterstaining) was not adjusted linearly (gamma 2) better visualizing the external morphology of the host. The figures present snapshots of the 3D-models, and snapshot resolution is not directly correlated with raw image resolution. General image manipulations (cropping, drawing, scaling) were executed with Fiji (28), and figures were built with the FigureJ module (29), which may resize pixels. Some large specimens of idotea were acquired as a mosaic of positions which were stitched together with the proprietary software of the microscope manufacturer (either LasX, Leica, or Zen, Zeiss). The figures S4D and 4E are Z-stack where the Depth of Focus had been extended by Sobel Projection from the transmitted light channel (CLIJ2 module, 30).

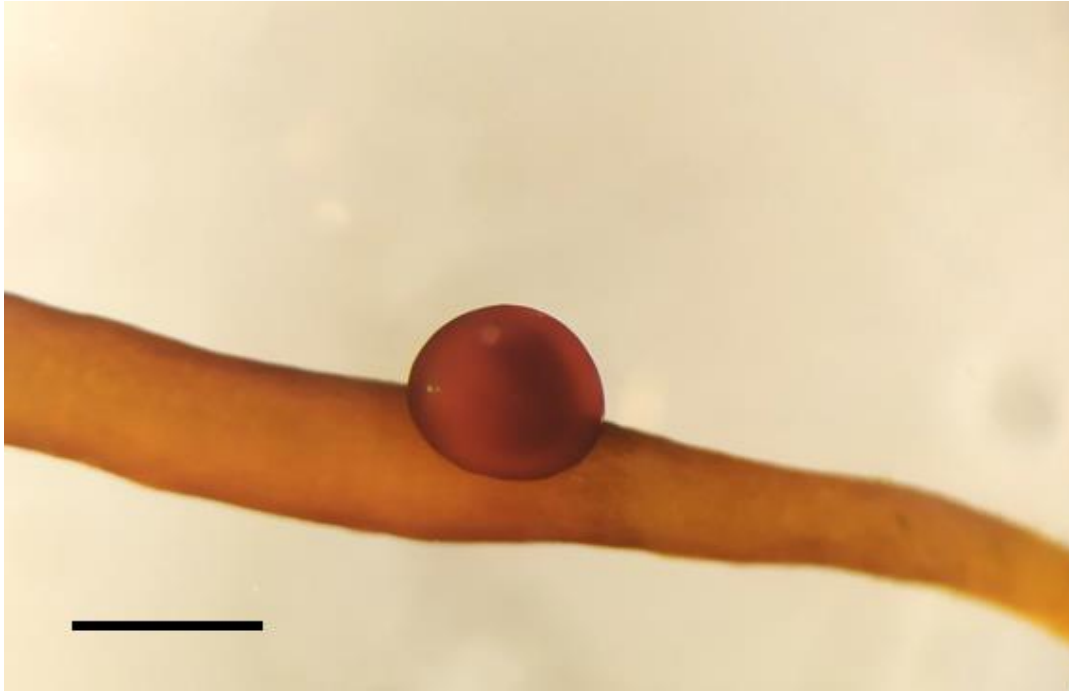


Fig. S1. Macroscopic view of a cystocarp on the surface of the *G. gracilis* female thallus

Fertilization occurs in the female thallus, where the zygote then develops into a diploid sporogenous tissue, protected and nurtured by the parental haploid female thallus. This complex structure called cystocarp corresponds to a single fertilization event. The scale bar is 1mm.

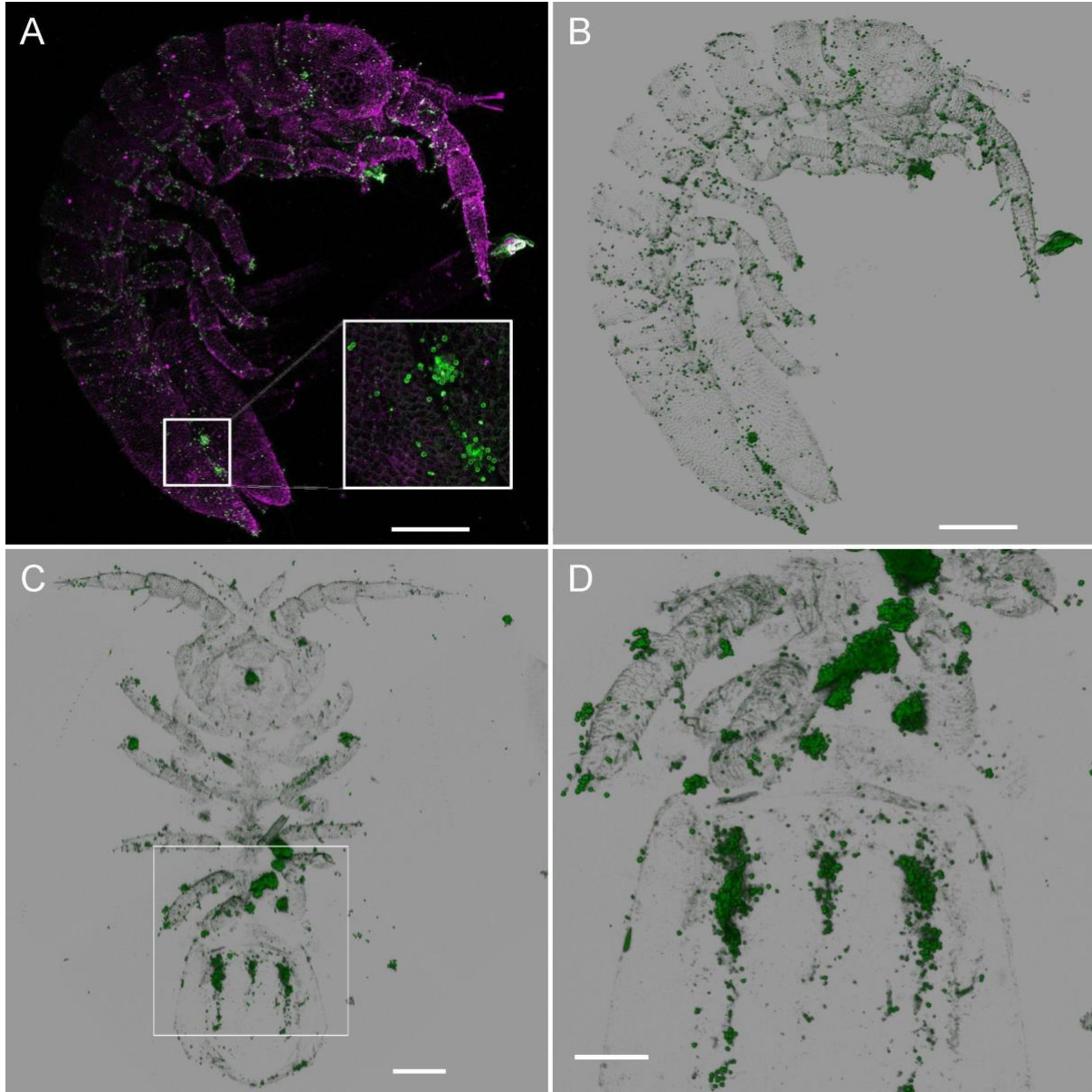


Fig. S2. Other views of *I. balthica* harboring *G. gracilis* male gametes (3D reconstructions of fixed specimens).

The sperm of *G. gracilis* are distributed over the entire surface of the body and aggregate mainly on the periopods, pleopods, and around the joints of exoskeleton segments. **(A)** A maximum projection rendering of the specimen with a close-up on the tail (pleopods). **(B)** A solid rendering of the green channel reveals the sperm and a limited staining of the chitinous teguments of the host by ConA-AF488. **(C)** Ventral views of another specimen. **(D)** Close-up of C-inset. The 3D model was reconstructed from a mosaic of CLSM Z-stack acquisitions with a 20x water immersion objective. Scale bars are 200µm in panels **(A)**, **(B)**, **(C)**, and 100µm in panel **(D)**

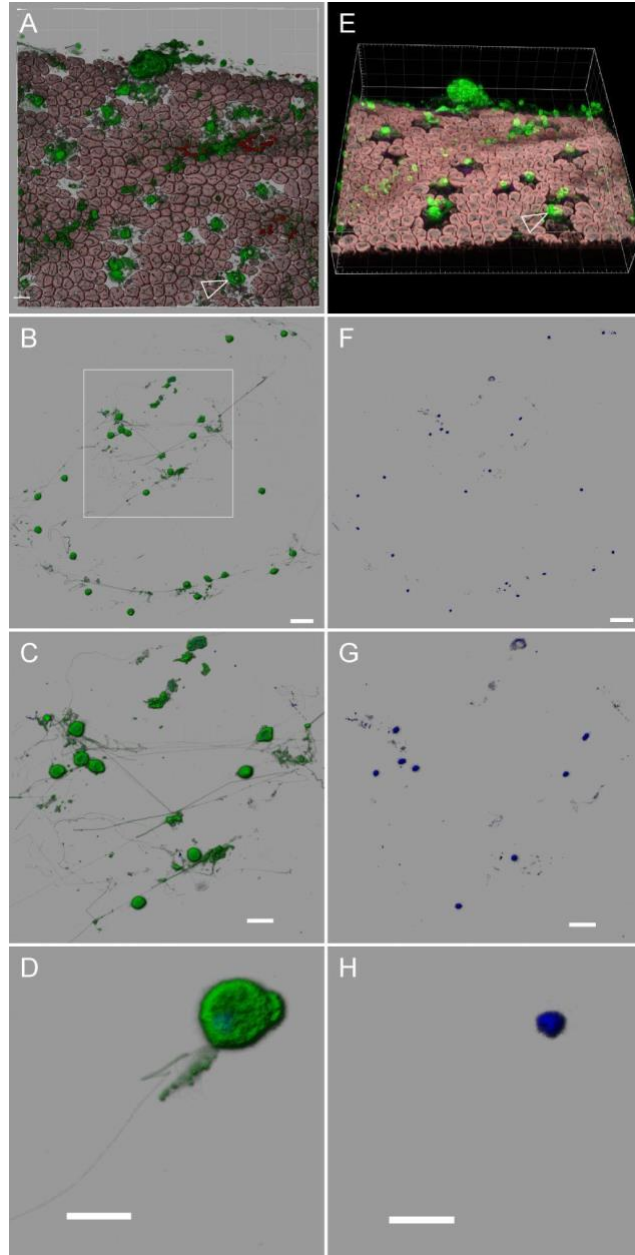


Fig. S3. The male gametes of *G. gracilis* are produced and released at the surface of the thallus.

(A) and (E) (tilted view) is a 3D reconstruction of a surface area from a live specimen of male gametophyte of *G. gracilis* imaged by means of CLSM. They illustrate the release of male gametes (ConA-AF488 staining, green) at the surface of the thallus (chloroplasts of cortical cells are pink as chlorophyll fluorescence, red, colocalizes with the phycoerythrin fluorescence, cyan). (B), (C) and (D): The mucilage surrounding the gametes is stained with ConA-AF488 (green), and it is also detected at the surface of the male conceptacles. These sites are depigmented since spermatia, and spermatangial parent cells do not show active chloroplasts. Arrows indicate conceptacle ostioles (in green). There is an “empty” space between cortical cell chloroplasts and these greenish spots

since the external cell wall is not visible. The spermatia show trailing mucilaginous appendages (mucus extensions) visible in **(B)**, **(C)**, and **(D)** (gametes alive). **(F)**, **(G)** and **(H)**: The staining of the DNA with Hoechst 33342 (blue) reveals the nuclei. **(D)** typical spherical morphology of the spermatia, circa 4.5 μ m in diameter, with their noticeable bump and external trailing mucilaginous appendages. Scale bars are 10 μ m in panels **(A)**, **(E)**, **(B)**, **(F)**, **(C)**, **(G)**, and 5 μ m in panels **(D)** and **(H)**.

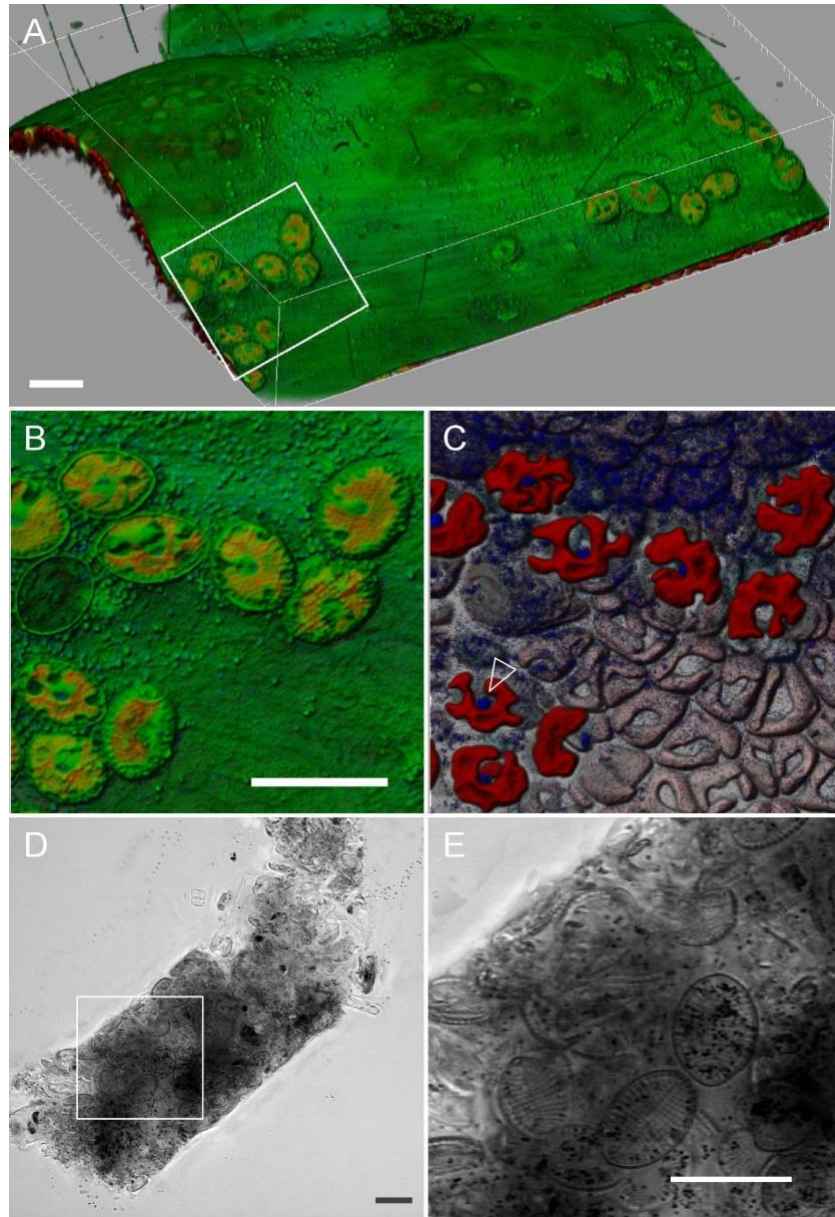


Fig. S4. Epiphytic diatoms on the red alga female contribute to the diet of the isopod.

Epiphytic organisms on the surface of the female thallus of *G. gracilis* sampled in the field (coccioid or filamentous bacteria, multicellular algae and diatoms). (A) Solid rendering of a 3D-reconstruction from a CLSM acquisition of a live specimen of female *G. gracilis*. The staining of the surface of the algae and cellular membranes of epiphytes (green) was achieved with DiOC6(3). Chloroplasts were localized from the fluorescence of chlorophyll (red) and/or phycoerythrin (cyan). Hoechst was used for staining DNA (Blue) with a limited diffusion into the cortical cells of *G. gracilis*. (B) Close-up of a diatom patch in A-inset showcases the presence of monoraphid diatoms. (C) Nuclei and chloroplasts (arrowhead) of diatoms are visible after removing the green channel. The chloroplasts of the cortical cells of *G. gracilis* look pinkish as they display both chlorophyll (red) and phycoerythrin (cyan) fluorescence. (D) Fecal pellets of *I. balthica* reared

with *G. gracilis* revealed a large number of diatom frustules (close-up **(E)** identified morphologically as *Cocconeis scutellum* (27). Scale bars are 20µm.

Table S1. Number of cystocarps, thalli length, and fertility success from each of the five replicates in the four experimental conditions. Total, mean and standard error (SE) of the number of cystocarps, thalli length and fertility success, and median fertility success are also given.

		Males and females	Males and females with idoteas	Females	Females with idoteas
<i>Replicate 1</i>	Number of cystocarps	2	24	0	3
	Thalli length (cm)	329.5	427.5	347.7	396.7
	Number of cystocarps/cm	0.01	0.06	0.00	0.01
<i>Replicate 2</i>	Number of cystocarps	6	83	0	3
	Thalli length (cm)	273.0	408.5	434.2	443.6
	Number of cystocarps/cm	0.02	0.20	0.00	0.01
<i>Replicate 3</i>	Number of cystocarps	43	634	2	38
	Thalli length (cm)	262.7	276.5	206.4	273.6
	Number of cystocarps/cm	0.16	2.29	0.01	0.14
<i>Replicate 4</i>	Number of cystocarps	3	338	0	84
	Thalli length (cm)	402.7	315.3	325.8	375.0
	Number of cystocarps/cm	0.01	1.07	0.00	0.22
<i>Replicate 5</i>	Number of cystocarps	0	182	0	50
	Thalli length (cm)	296.5	350.2	267.5	308.4
	Number of cystocarps/cm	0.00	0.52	0.00	0.16
<i>Total</i>	Number of cystocarps	54	1261	2	178
	Thalli length (cm)	1564.4	1778.0	1581.6	1797.3
	Number of cystocarps/cm	0.035	0.709	0.001	0.099
<i>Mean (SE)</i>	Number of cystocarps	10.8 (8.1)	252.2 (109.3)	0.4 (0.4)	35.6 (15.3)
	Thalli length (cm)	312.9 (25.2)	355.6 (28.2)	316.3 (38.4)	359.5 (30.6)
	Number of cystocarps/cm	0.040 (0.031)	0.829 (0.405)	0.002 (0.002)	0.108 (0.043)
<i>Median</i>	Number of cystocarps/cm	0.007	0.520	0.000	0.139

Table S2. Identities of males and females used in each replicate.

Replicates	Female identities	Male identities
1	32, 125, 173	3, 240, 54, 151, 20, 87, 9, 12, 61, 181
2	23, 30, 163	3, 240, 54, 151, 20, 87, 9, 12, 61, 181
3	32, 163, 249	3, 240, 54, 151, 20, 87, 9, 12, 61, 181
4	30, 86, 125	3, 240, 54, 151, 20, 87, 9, 12, 61, 181
5	23, 86, 163	3, 240, 54, 151, 20, 87, 9, 12, 61, 181

Movie S1. Movements of *Idotea balthica* in the aquarium during the experiment (20 seconds).

This video shows the aquarium containing males (on the left) and females (on the right) of *G. gracilis* with twenty *I. balthica* (see experiment Fig. 2A). The isopods of different sizes are foraging on the seaweed. Some of them are difficult to observe as they are firmly gripped onto the algae, and spermatia can thus deposit on the setae of their pereopods. The isopods move in the aquarium, creating water flow and transporting spermatia as they swim from male to female seaweeds.

Movie S2: 3D animation from CLSM images of *I. balthica* harboring *G. gracilis* male gametes (fixed specimen, 33 seconds)

The 3D model has been reconstructed from a confocal laser scanning microscopy (CLSM) acquisition of a young specimen of *I. balthica* collected on a reproductive-male gametophyte of *G. gracilis* (tegument of the isopod was fixed and stained with calcofluor white (CFW) and the spermatia with ConA-AF488. This video illustrates the distribution of spermatia of *G. gracilis* onto the isopod body. They are observed especially on the parts of the animal that are in contact with the algae (i.e., the setae of pereopods).