Widespread transmission of independent cancer lineages within multiple bivalve species

Michael J. Metzger1,2, Antonio Villalba3,4, María J. Carballal5, David Iglesias3, James Sherry5, Carol Reinisch5, Annette F. Muttray6,7, Susan A. Baldwin6 & Stephen P. Goff1,2,8

Most cancers arise from oncogenic changes in the genomes of somatic cells, and while the cells may migrate by metastasis, they remain within that single individual. Natural transmission of cancer cells from one individual to another has been observed in two distinct cases in mammals (Tasmanian devils1 and dogs2,3), but these are generally considered to be rare exceptions in nature. The discovery of transmissible cancer in soft-shell clams (Mya arenaria)4 suggested that this phenomenon might be more widespread. Here we analyse disseminated neoplasia in mussels (Mytilus trossulus), cockles (Cerastoderma edule), and golden carpet shell clams (Polititisipes aureus) and find that neoplasias in all three species are attributable to independent transmissible cancer lineages. In mussels and cockles, the cancer lineages are derived from their respective host species; however, unexpectedly, cancer cells in P. aureus are all derived from Venerupis corrugata, a different species living in the same geographical area. No cases of disseminated neoplasia have thus far been found in V. corrugata from the same region. These findings show that transmission of cancer cells in the marine environment is common in multiple species, that it has originated many times, and that while most transmissible cancers are found spreading within the species of origin, cross-species transmission of cancer cells can occur.

Disseminated neoplasia, or haemoc neoplasia, a leukaemia-like disease, occurs with high prevalence in multiple bivalve species4,6. Here we investigate the possibility that cancers in three species could be attributed to transmissible cancer cells, and whether these cancers are restricted to the species of origin or can undergo cross-species transmission.

Mussels (M. trossulus; Fig. 1a) are subject to disseminated neoplasia in the Pacific Northwest Coast4–7, and evidence of common polymorphisms in neoplasias suggested that these might represent a transmissible cancer8. Twenty-eight mussels (M. trossulus) collected from West Vancouver were screened for neoplasia by analysing haemocytes and haemocytosis for the rounded, non-adherent morphology of neoplastic cells. Two were identified with high levels of neoplastic cells. We sequenced part of the mitochondrial cytochrome c oxidase I (mitCOI) gene in host tissue and neoplastic haemocytes from the two diseased animals and four normal animals to test whether the neoplastic cells were derived from the host individuals or exhibited a distinct genotype, the hallmark of transmissible neoplasia. While solid tissue and haemocyte genotypes within each normal animal were always identical, solid tissue and neoplastic haemocyte genotypes of the diseased animals were discordant (Fig. 1b and Extended Data Fig. 1). Moreover, the same single nucleotide polymorphisms (SNPs) were found in neoplastic cells of the two diseased individuals, indicating that the cancer cells were not of host origin and suggesting that they arose from a single clonal origin. EF1α gene sequences also revealed that the genotypes of the host cells and neoplastic haemocytes were discordant, and that the genotypes of the neoplastic cells of the two different animals were again identical to each other (Fig. 1c).

To determine whether this transmissible cell line was widespread in the M. trossulus population, 250 were collected from Vancouver Island, and seven potentially diseased individuals were analysed. In one neoplastic sample (MW81), the haemocyte and tissue genotypes did not match, and the haemocytes contained the same mitCOI allele and the same EF1α major and minor alleles found in the other samples (Fig. 1b, c and Extended Data Fig. 1). These genotypes strongly indicate the existence of a M. trossulus-derived transmissible cancer lineage circulating in the wild population.

High prevalence of disseminated neoplasia has been observed in two species of bivalves on the Galician Coast: cockles (C. edule)10,11 and golden carpet shell clams (P. aureus, previously named Venerupis aurea)12. The disease in cockles (C. edule) exhibits one of two distinct morphologies, termed types A and B13,14. We collected about 150 cockles (C. edule; Fig. 2a), and examined the genotypes of solid tissue and haemocytes of six normal individuals and six with high (75–90%) or moderate (15–75%) amounts of neoplastic cells in the haemolymph. Nine polymorphic microsatellite loci15 were amplified from normal animals, and allele sizes from tissue and haemocytes of each normal animal all matched, but allele sizes in haemocytes and tissue of diseased animals were discordant (Fig. 2b, c). In a phylogenetic tree based on microsatellite alleles16,17 the neoplastic haemocyte genotypes did not group with the host tissue genotypes, consistent with transmissible cancer, and instead clustered into two distinct branches, suggesting two independent cancer lineages (Fig. 2d). We sequenced the mitCOI gene and identified several SNPs that were present only in lineage 2, and not in any of the normal animals. No unique SNPs were identified in the mitCOI region sequenced in lineage 1.

The microsatellite alleles and mitCOI SNPs suggest two independent cancer lineages, but these data are also consistent with two subgroups that have diverged from a single transmissible cancer lineage. To investigate this question, we sequenced an approximately 3-kilobase intron-spanning region in EF1α from six normal individuals and two diseased individuals from each lineage (Fig. 2e). Both neoplastic haemocyte alleles were different from the tissue alleles in all diseased individuals, and the two alleles of the neoplasm in each diseased individual were nearly identical to the two alleles of the other individual in the lineage. However, both alleles in lineage 1 cells were different from those in lineage 2. Moreover, the neoplastic alleles were more closely related to some normal alleles than they were to the alleles of the alternative lineage. These data strongly suggest an independent origin of these two lineages.

Histological and morphometric examination showed that all three samples in lineage 1 were type A (characterized by a looser arrangement of neoplastic cells in the connective tissue, with pleomorphic nuclei) and all three samples in lineage 2 were type B (tighter arrangement,
Figure 1 | Analysis of tissue and haemocyte genotypes of normal mussels (M. trossulus) and mussels with disseminated neoplasia using mitochondrial and nuclear DNA markers. a. Representative M. trossulus, with ruler for scale. b. Sequencing of mtCOI (bases 99–759) was conducted for both host solid tissue (T) and haemocytes (H) of normal (*n = 4*) and neoplastic mussels (*n = 3*). Open boxes mark SNPs that differ from allele A. Filled boxes mark discordance between neoplastic haemocyte and neoplastic mussels (*for both host solid tissue (T) and haemocytes (H)* of normal (*with ruler for scale.*). Attempts to transfer M. arenaria indicates that the cancer spreading in the species is not limited to the M. arenaria but also affects other bivalves in the same habitat. We previously identified an LTR-retrotransposon, Steamer, which was amplified in the transmissible neoplasia lineage in the soft-shell clam (M. arenaria). Using degenerate primers, we identified at least one Steamer-like element (SLE) in each of the three species studied here. In each case, copy numbers were variable among individuals, but no massive amplification of these particular retrotransposons was observed in any of the transmissible cancers assayed (Extended Data Table 1). This suggests that Steamer-like retrotransposon amplification is not essential in development of transmissible clones. Our results indicate that transmission of contagious cancer cells is a widespread phenomenon in the marine environment, with multiple independent lineages developing in multiple species in four bivalve families. Along with the recent identification of a second independent lineage of transmissible cancer in Tasmanian devils, these findings confirm that, under suitable conditions, the development of transmissible cancer can occur in multiple species, and suggests that some species may be more susceptible to development of transmissible neoplasia than others.
of disease to *M. arenaria*[^36], and injection of *M. trossulus* cancer cells into multiple bivalve species only resulted in engraftment in *M. trossulus*[^27]. Our finding that multiple cancer lineages are most often found to spread within the original host species is consistent with these previous experiments, and suggests that there may be species-specific restriction factors that prevent engraftment into divergent hosts.

In this context, our observation of cross-species transmission, a cancer from one species spreading through another, is particularly striking. They both belong to the Veneridae family (some studies suggest that *P. aureus* should belong to the Venerus genus[^28]), and coexist in the same beds. This close relationship may have aided the transmission.

It is notable that despite ongoing surveillance of the bivalves of the Galician coast since the 1990s, only one *V. corrugata* has been identified as harbouring disseminated neoplasia (S. Darriba, personal communication), while the prevalence of disease in *P. aureus* is quite high[^12] (12% of the individuals collected for the current study had high levels of neoplasia and 42% had some detectable level of disease). This would be explained if the cancer originated in *V. corrugata*, led to selective loss of susceptible animals, and left the current population of *V. corrugata* resistant to engraftment and disease. The spread of transmissible cancers and the evolutionary pressure to defend against them would be explained if the cancer originated in *V. corrugata*. 

[^36]: S. Darriba, personal communication.
[^27]: S. Darriba, personal communication.
[^28]: S. Darriba, personal communication.
In summary, our findings show that transmission of cancers between individuals within a species may be more common than previously assumed, and that transmission can even occur between species. We now know of eight transmissible cancers in nature: one lineage in dogs, two lineages in Tasmanian devils, and five lineages circulating in four species of molluscs, including one example of cross-species transmission. Further studies may well reveal additional examples. The remarkable ability of tumours to acquire new phenotypes that promote their own survival and propagation.

Online Content Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper; references unique to these sections appear only in the online paper.

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Author Contributions
M.J.M. and S.P.G. wrote the manuscript. M.J.M. conducted molecular analyses. A.F.M. and S.A.B. collected and diagnosed M. trossulus from West Vancouver. J.S. and C.R. collected and diagnosed M. trossulus from Vancouver Island. D.I., M.J.C., and A.V. collected and diagnosed C. edule and P. aureus. M.J.C. produced micrographs of C. edule neoplastic haemocytes, and D.I. conducted morphometric analysis.

Author Information
Sequences generated in this work have been deposited in GenBank under accession numbers KX018521–KX018605. Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of the paper. Correspondence and requests for materials should be addressed to S.P.G. (spg1@cumc.columbia.edu).

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METHODS

No statistical methods were used to predetermine sample size. The experiments were not randomized. The investigators were not blinded to diagnosis during sequencing and morphology assessment.

Collection and diagnosis of M. trossulus. Mussel (M. trossulus) specimens 5–6 cm long were collected from the intertidal zone at low tide (noon) on 18 April 2015 at Copper Beach (49° 22′ 41″N, 123° 16′ 44″ W, West Vancouver, British Columbia, Canada). They were transported to the laboratory in aerated seawater from Copper Beach. Total body mass at laboratory, 0.5–1.0 ml haemolymph cell was removed from the posterior adductor muscle. For each individual, one drop of haemolymph was placed on a poly-L-lysine-coated slide and let sit for 10–15 min to allow the cells to spread and attach to the slide. Thereafter, the slide was viewed under a Zeiss Axiosstar light microscope at ×40 magnification. Normal (non-neoplastic) specimens were those with greater than 90% cells with normal appearance: that is, agranular or granular haemocytes with spread pseudopodia. Fully leukaemic (diseased) specimens were those with prolific amounts (>90%) of round, non-adherent cells. Haemolymph was added to 1.5 ml Eppendorf tubes and spun at 900g for 3 min. After the supernatant was withdrawn, the cells were re-suspended in absolute ethanol before shipping. Excised tissues (mantle, foot, and gills) were preserved in absolute ethanol before shipping. Of 28 individuals collected from West Vancouver, two had high levels of neoplastic disease.

A second set of M. trossulus samples were collected from Esquimalt, Vancouver Island, British Columbia, Canada. Haemolymph was extracted and cell morphology was used for diagnosis as above. Haemocyte and solid tissue samples were fixed in ethanol before DNA extraction. Of 250 individuals collected from Esquimalt, 9 were scored as potentially moderately or highly diseased, with 7 samples available for analysis.

Collection and diagnosis of C. edule and P. aureus. Cockles (C. edule) were collected from an intertidal bed named O Sarrido (42° 30′ N, 8° 49′ W) and golden carpet shell clams (P. aureus) and pullet shell clams (V. corrugata) were collected from a subtidal bed named O Bohído (42° 32′ N, 8° 51′ W); both shellfish beds are located in the ria of Arousa (Galicia, northwest Spain). Once in the laboratory of Centro de Investigaciónes Marinas, each cockle and clam was notched through the shell margin close to the posterior adductor muscle and haemolymph (as much as possible) was collected from the posterior adductor muscle using a 2-ml syringe with a 21-gauge needle. A small quantity (about 100 μl) of haemolymph was used to produce a cell monolayer on a slide by cytocentrifugation (92g, 5 min, 4°C), which was fixed, stained with a Hemacolor (Merck) kit, and examined with light microscopy for diagnosis of disseminated neoplasia; the remaining haemolymph was preserved in absolute ethanol for molecular analysis. After haemolymph collection, molluscs were shackled and a small piece of mantle (about 5 mm × 5 mm) was removed and preserved in absolute ethanol for molecular analysis; additionally, an approximately 5-mm-thick section of meat, containing gills, visceral mass, foot, and mantle lobes, was fixed in Davidson’s solution and embedded in paraffin. Sections of 5-μm thickness were stained with Harris’s haematoxylin and eosin [19]. Histological sections were examined under light microscopy for histopathological analysis.

Morphometric analysis was conducted on histological sections of cockle (C. edule) samples. Neoplastic cells (both A and B types) had a unique morphology, and were clearly distinguished from normal cells, with much larger overall size, and much larger nucleus. The longest diameters of the cell and the nucleus of at least ten neoplastic cells for each individual were measured by direct examination of histological sections with light microscopy using a reticle. Two-tailed t-tests were used for comparisons of morphometric data from different individuals and types.

Cockles (C. edule) and golden carpet shell clams (P. aureus) were ranked according to a scale of disease severity based on haemolymph diagnosis: non-affected (N0; or N); low severity (N1, or L), when individuals showed proportions of neoplastic cells lower than 15% and were represented, all haemolymph; moderate severity (N2, or M), when the proportion ranged from 15% to 75%; and high severity (N3, or H), when the proportion was higher than 75% (ref. 32). Seventy-four golden carpet shell clams (P. aureus) were collected with 12 diagnosed with light, 10 with moderate, and 9 with heavy neoplasia. About 150 cockles (C. edule) were collected and a subset was analysed both for disease and for morphological type of neoplastic cells. Of the 30 in this subset, two had type A neoplastic cells (one light and the other moderate) and one had low levels of type B neoplastic cells. We have also collected and analysed hundreds of pullet clams (V. corrugata) from multiple beds throughout Galicia from 1988 to the present, including about 100 from the same bed in which the samples of P. aureus were collected for this study (O Bohído). So far, we have not observed any V. corrugata samples with neoplastic disease. One V. corrugata individual from a different location has been identified by a different researcher as harboursing disseminated neoplasia, with a different morphology than that observed in P. aureus neoplasia (S. Darriba, personal communication).

DNA extraction. DNA was extracted from ethanol-fixed haemocytes using DNeasy Blood and Tissue Kit (Qiagen). DNA extraction of tissues used the same kit, but included an additional step to reduce the amount of PCR-inhibiting polysaccharides. After tissue lysis, 63 μl of buffer P3 was added to lysate and allowed to precipitate for 5 min. Lysate was spun 10 min at full speed at 4°C, and the resulting supernatant was mixed with buffer AI for 10 min at 37°C, then mixed with ethanol and added to the column, continuing with the standard protocol.

mtCOI, EF1α, ITS, Steamer-like PCR. Primers and annealing temperatures used are presented in Table 2. PCR products were cloned using the pGEM-T Easy Vector System (Promega) polymerase (Agilent) was used to amplify 10 ng of genomic DNA for 35 cycles. Extension was at 72°C for 15 s for mtCOI for all bivalves [13]. For P. aureus, ITS was amplified (using primers modified from refs 34, 35) with extension for 30 s. Despite multiple copies of ITS in genomic DNA, a single sequence was observed for each normal P. aureus and V. corrugata, and a single pair of host/neoplasm sequences was observed in each diseased individual. PCR for EF1α followed the same program, with extension for 20 s in mussels (M. trossulus) and clams (P. aureus and V. corrugata) and 1 min 30 s in cockles (C. edule). For amplification of Steamer-like elements, a degenerate primer pair was designed to match conserved regions in reverse transcriptase and integrase of the LTR-retrotransposon, Steamer, and 10 ng of DNA was amplified for 35 cycles, annealing at 55°C for 20 s and extending for 1 min at 72°C. PCR products were directly sequenced. When multiple alleles could not be resolved by direct sequencing, PCR products were cloned using the Zero Blunt TOPO Kit (Invitrogen), and at least six colonies were sequenced. Sequences were aligned with Clustal W with some manual adjustment. Primer-binding regions were excluded from analysis and are not included in the sizes listed as sequenced. Maximum likelihood phylogenetic trees were generated using PhyML 3.0 (ref. 36), using the HKY+I substitution model, with 100 bootstrap replicates, treating gaps in the alignment as missing data. Trees were visualized using FigTree version 1.3, with addition of markers at the branch termini. Each phylogenetic tree based on alignment of sequence at a single locus (Figs 1c, 2b and 3b–d) includes a scale bar that shows genetic distance (0–1) based on the frequency of nucleotide divergence. All sequences are available in GenBank (accession numbers KX018521–KX018605).

Analysis of microsatellite loci from C. edule. Microsatellites were amplified using primers for 12 loci, reported previously to be polymorphic in cockle (C. edule) populations [15]. Of the 12 primer pairs, 9 pairs amplified products in all cockle DNA samples, with 1–2 alleles observed in normal samples and 1–4 neoplastic alleles observed. These were used in all further analyses, with fluorescent modifications on the 5′ end of the forward primers as listed (Extended Data Table 2). KOD polymerase (Millipore) was used to prevent ambiguity of untemplated addition of A residues by Taq polymerase. Products were run on an agarose gel for visualization.

Allele sizes were identified to single-base precision by fragment analysis using fluorescent primers (6-FAM, PET, NED, and VIC) using a 3730xl Genetic Analyzer with the LIZ-500 size standard (Applied Biosystems, operated by Genewiz). Peaks were called using Peak Scanner 2.0 (Applied Biosystems), rounding sizes to whole bases, with some manual adjustment to keep alleles of the same size together.

We used the R package Poppr [17] to generate distance matrices and phylogenetic trees using Bruvo’s method with the infinite alleles model. The value of c (repeat size) was set to 0.00001 to calculate distances using the band sharing model, in which alleles are either identical distances (distance = 0) or non-identical (distance = 1). Each allele was coded as a dominant marker [17], with a single variable for each allele observed at each position (104 total markers from 9 loci). For each allele (a single size at a single locus), a sample is observed to be present, absent, or the information missing. For example, a sample with sizes 195 and 199 at locus A would be marked as ‘present’ for marker ‘A-195’ and ‘A-199’, but ‘absent’ for all other sizes at that locus (like ‘A-203’ and ‘A-207’). This analysis method ignores the uncertainty in the copy number of each locus in aneuploid cancer cells and allows for ambiguity at particular alleles. In cases where the haemocyte and tissue genotypes could be distinguished, all alleles were scored as the average of the pairwise differences at all allele sizes observed (104 pairwise comparisons if no data are missing). In pairwise comparisons for each allele, two samples either have a distance of 0 (meaning that the particular allele size is either present in both samples or absent in both samples) or a distance of 1 (meaning that the particular allele size is present in one sample but absent in the other). If data are missing for one or both, then that specific comparison is dropped with no contribution to the total distance between the two samples. The genetic distances calculated are based on the alleles observed at the nine loci, so the absolute value
of the scale itself is therefore dependent on the number of observed alleles that are included in the analysis. Source data for the generation of the phylogenetic tree lists all observed alleles coded as present (1), absent (2), or missing (0) for each sample.

As an alternative analysis method, the data were analysed using Bruvo’s method as polyploid data with nine loci, using only the individuals that could be confidently identified. In this alternative analysis, the topology of the resulting tree was not different—all nodes with bootstrap values above 50 were maintained. In this analysis, the genetic distance between the representatives of lineage 1 (H3) and lineage 2 (H1) was 0.676, and the distances to the closest normal sample were 0.595 (H3 to N5) and 0.648 (H1 to N1 and N2).

**qPCR.** Species-specific qPCR of the EF1α locus in *P. aureus* and *V. corrugata* was done using FastStart Universal SYBR Green Master Mix (Roche). Primers were designed to amplify the same region, with the 3′ end of both forward and reverse primers on sites that differ between the two species (Extended Data Table 2). Standard curves for each primer set were generated using two control plasmids (one containing the *P. aureus* EF1α fragment and *P. aureus* SLE fragment, the other containing the fragments from *V. corrugata*). EF1α fragments were amplified using conserved primers (Extended Data Table 2). Fragments were cloned with a Zero Blunt TOPO Kit (Invitrogen), and plasmids were linearized with NotI before qPCR. Standard curve samples (10^4–10^7 copies per reaction) and experimental samples (2.5 ng per reaction) were done in triplicate. No amplification was detected with the *P. aureus* primers on the *V. corrugata* plasmid or with the *V. corrugata* primers on *P. aureus* plasmid (using up to 10^7 copies per reaction). The fraction of *V. corrugata* in each sample was calculated as the copy number using *V. corrugata*-specific primers divided by copy number of *P. aureus*– plus *V. corrugata*-specific amplification.

Quantification of Steamer-like elements in the three species was performed by the same method, using primers in the RT-IN region of the retrotransposon (on the basis of sequence obtained through amplification using degenerate primers) and control primers amplifying a region in EF1α. All primers used to amplify SLEs and EF1α from genomic DNA for cloning into standards and primers for qPCR are listed in Extended Data Table 2. For each species, a single control plasmid containing both the EF1α and SLE fragments was used.

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Extended Data Figure 1 | Analysis of mtCOI amplified from tissue and haemocyte DNA of normal and diseased mussels (M. trossulus).

A partial region of the mtCOI gene was amplified from genomic DNA of solid tissue and haemocytes from mussels (M. trossulus) and directly sequenced (Fig. 1b). Trace images show a region flanking a representative SNP marked with an open triangle (tissue) or closed triangle (haemocytes). a, b, In normal mussels, tissue and haemocyte alleles match (with G496 at all positions). c–e, In mussels with disseminated neoplasia, the tissue and haemocyte alleles are different. Neoplastic haemocytes have A at position 496 and G in tissue, with some A observable in tissue, probably because of infiltration of neoplastic haemocytes.
Extended Data Figure 2 | Quantification of Steamer-like element genomic copy number in mussels (*M. trossulus*), cockles (*C. edule*), and golden carpet shell clams (*P. aureus*). a–d, Fragments from the SLE reverse transcriptase region and EF1α genes were cloned from each species. Haploid copy numbers of Steamer-like elements (SLE) were quantified by determining the ratio of SLE/EF1α in genomic DNA from haemocytes. Single species-specific SLEs were analysed in (a) mussels (*M. trossulus*) and (b) cockles (*C. edule*). c, d, In golden carpet shell clams, one SLE (SLE-Pa) was cloned from a normal *P. aureus* (clam N2) and a different one (SLE-Vc) was cloned from neoplastic cells (clam H2). Both SLEs could be found in both species, and qPCR analysis confirmed that SLE-Pa is more highly amplified in *P. aureus* and has fewer copies in *V. corrugata* and in the neoplastic cells derived from *V. corrugata.*
### Extended Data Table 1 | Morphometric analysis of type A and type B cockle (C. edule) neoplasia

| Cockle ID | Type | Cells counted (N) | Cell diameter ± SD (µm)* | Nuclear diameter ± SD (µm)† | Nuclear/cell ratio ± SD‡ |
|-----------|------|------------------|--------------------------|-----------------------------|--------------------------|
| M1        | A    | 15               | 9.0 ± 1.2                | 6.7 ± 1.0                   | 0.75 ± 0.09               |
| H2        | A    | 15               | 8.7 ± 0.9                | 6.6 ± 0.8                   | 0.76 ± 0.06               |
| H3        | A    | 10               | 8.5 ± 1.1                | 6.5 ± 1.0                   | 0.77 ± 0.07               |
| total     | A    | 40               | 8.8 ± 1.0                | 6.6 ± 0.9                   | 0.76 ± 0.07               |
| M2        | B    | 10               | 7.7 ± 0.7                | 5.5 ± 0.5                   | 0.72 ± 0.07               |
| M3        | B    | 15               | 6.7 ± 1.2                | 4.5 ± 0.6                   | 0.70 ± 0.15               |
| H1        | B    | 15               | 7.3 ± 1.0                | 5.4 ± 0.8                   | 0.75 ± 0.09               |
| total     | B    | 40               | 7.2 ± 1.1                | 5.1 ± 0.8                   | 0.72 ± 0.11               |

*Cell diameter is statistically different between types A and B (P < 0.0001). All tests are two-tailed t-tests.
†Nuclear diameter is statistically different between types A and B (P < 0.0001); for all pairwise comparisons between type A and type B individuals, P < 0.05.
‡Ratio of nuclear diameter to cell diameter is not significantly different between types.
## Extended Data Table 2  Primers used in PCR and qPCR

| Target | Forward Primer | Reverse Primer | Temp |
|--------|----------------|----------------|------|
| **mCOI** | LCO1490 | HCO2198 | 50°C |
| **ITS** | ITS-3d | ITS-4r | 55°C |

### EF1α

| Mussel (M. trossulus) | consEF1-F1 | MEF1-R2 | 50°C |
|-----------------------|------------|---------|------|
| Cockle (C. edule)    | CeEF1-F2  | CeEF1-R3 | 55°C |
| P. aureus & V. corugata | consVEF1F3b | consVEF1R3b | 55°C |

### Cloning qPCR Standards

#### EF1α

| Mussel (M. trossulus) | consEF1-F1 | consEF1R2 | 50°C |
|-----------------------|------------|-----------|------|
| Cockle (C. edule)    | CeEF1-F1  | CeEF1-R3  | 50°C |
| P. aureus & V. corugata | consVEF1F3b | consVEF1R3b | 50°C |

### SLE

| Mussel (M. trossulus) | DHKPL-F1 | PXRPW-R1 | 55°C |
|-----------------------|----------|----------|------|
| Cockle (C. edule)    | LVW-F1   | PXRPW-R1 | 55°C |
| P. aureus & V. corugata | DHKPL-F1 | PXRPW-VaR1 | 55°C |

### qPCR

| Target | Forward Primer | Reverse Primer | Control Plasmids |
|--------|----------------|----------------|-----------------|
| EF1α (M. trossulus) | MEF1qF2 | MEF1qR1 | pMEF1-SLE4 |
| Cockle (C. edule)    | CeEF1qF2 | CeEF1qR2 | pCockleSLE-EF1 |
| P. aureus | VaEF1qF1 | VaEF1qR1 | pVan2-EF1SLE |
| V. corugata | VeEF1qF1 | VeEF1qR1 | pVan2-EF1SLE |

### SLE

| SLE-Mt | MsLEqF2 | MsLEqR1 | pMEF1-SLE4 |
|--------|---------|---------|------------|
| SLE-Ce | cockleRT-F2 | cockleRT-R2 | pCockleSLE-EF1 |
| SLE-Pa | VaNSLEqF1 | VaNSLEqR4 | pVan2-EF1SLE |
| SLE-Vc | VaHLEqF1 | VaHLEqR1 | pVan2-EF1SLE |