Pallenopsis patagonica specimen and outgroups with accessions, from Table 1, Domel et al, in review, Frontiers in ... (Antarctic Inverts project)

Website: https://www.bco-dmo.org/dataset/671948
Data Type: Cruise Results
Version: 1
Version Date: 2016-12-27

Project
» Genetic connectivity and biogeographic patterns of Antarctic benthic invertebrates (Antarctic Inverts)

Contributors	Affiliation	Role
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Abstract
This dataset was published as Table 1 from Dömel et al (2017). It contains DNA sequence data from Pallenopsis patagonica (Pallenopsidae: Pycnogonida) collected from specimens found in the Southern Ocean.

Table of Contents

Coverage

Spatial Extent: **N**:-43.393 **E**:169.965 **S**:-77.016 **W**:-175.093
Temporal Extent: 2013-01-06 - 2013-12-20

Methods & Sampling

From Dömel et al (2017):

2.1 Specimens and sampling sites
For the remainder of this study, we use the term Pallenopsispatagonica sensu lato (s.l.) when referring to the whole species complex including P. yepayekae, because it groups within clades morphologically originally identified as P. patagonica. Individuals of P. patagonica s.l. from the shelf of South America, Subantarctic islands as well as around the Antarctic continent were analyzed (Table 1, Figure 1). Chilean specimens were collected by divers during Huinay Fjordos expeditions (HF16, HF21, HF24 and HF26). Falkland samples (ZDLT1) were provided by Vladimir Laptikhovsky (Falkland Islands Fisheries Department, Stanley, Falkland Islands). Samples from the Southern Ocean were collected using different bottom trawls during several cruises on board the RRS James Clark Ross (British Antarctic Survey, Cambridge, UK) and the RV Polarstern (Alfred Wegener Institute Helmholtz Center for Polar and Marine Research, Bremerhaven, Germany). After collection, specimens were stored in ethanol (96%). Specimens were morphologically inspected and assigned to P. patagonica s.l. before being molecularly studied.

2.2 Molecular analyses

Muscle tissue was extracted from the tibia using sterile scalpel and forceps. DNA was isolated from the tissue using a modified salt precipitation protocol after Sunnucks & Hales (1996) (see Weiss & Leese 2016). Extracted DNA was eluted in 100 ul TE minimum buffer (0.1xTrishydrochloride buffer, pH 8.0, containing 0.1 mM EDTA). The amplification of the mitochondrial cytochrome c oxidase subunit I gene (COI) and a ribosomal gene region covering the 18S-ITS1-5.8S-ITS2-28S stretch (internal transcribed spacer, ITS) was carried out in 25 ul reactions containing 1x (2.5 ul) PCR buffer (5Prime), 0.2 mM dNTPs, 0.5 uM of each primer, 0.025 U/ul (0.125 ul) Hotmaster Taq (5Prime) and 1 ul template DNA, topped up to 25 ul with sterile water. A 658 bp long fragment of the COI was amplified using the common barcoding primer pair HCO2198 and LCO1490 (Folmer et al. 1994). The optimal temperature profile for the PCRs with these primers was an initial denaturation at 94 deg.C for 2 minutes, followed by 36 cycles of denaturation at 94 deg.C for 20 seconds, annealing at 46 deg.C for 30 seconds, extension at 65 deg.C for 60 seconds, and a final extension at 65 deg.C for 7 minutes. For ITS, an approximate 1000 bp long fragment was amplified using primers ITSRA2 and ITS2.2 (Arango & Brenneis 2013). PCR cycling program was initial denaturation at 94 deg.C for 3 minutes, followed by 35 cycles of denaturation at 94 deg.C for 30 seconds, annealing at 55 deg.C for 75 seconds, final extension at 65 deg.C for 5 minutes.

For sequencing, 10 U (0.5 ul) Exonuclease I (Thermo Scientific), 1.5 U (1 ul) FastAP Thermosensitive Alkaline Phosphatase (Thermo Scientific) and 9 ul PCR product per reaction were used. The purification mix was incubated for 25 minutes at 37 deg.C, followed by a denaturation step at 85 deg.C for 15 minutes. For sequencing at GATC Biotech AG (Cologne, Germany) 5 ul of purified PCR product was mixed with 5 ul of 5 pmol/ul primer. Forward and reverse primers were used to sequence both directions of the DNA strands.

For ITS sequences of samples reported by Harder et al. (2016) (herein labeled with GenBank numbers starting with KT98) DNA extraction was performed as stated in Harder et al. (2016). For ITS amplification the same primer pair as mentioned above was used. PCR mixture consisted of 1x PCR buffer, 0.75 U Taq DNA polymerase (5Prime), 2.5 mM Mg2+, 10 nmol of each dNTP, 1 ul of template DNA, 0.5 uM of each primer, and water to 25 ul. PCR cycling program was run, with an initial denaturation at 94 deg.C for 2 minutes, followed by 37 cycles of denaturation at 94 deg.C for 20 seconds, annealing at 55 deg.C for 30 seconds, extension at 65 deg.C for 80 seconds, with a final extension at 65 deg.C for 10 minutes. Successful amplification was confirmed by visualizing PCR products on a 1% agarose gel stained with ethidium bromide. Target PCR product was gel extracted and purified using a Qiagen QIAquick® Gel Extraction Kit according to the manufacturer's recommendations. Bidirectional Sanger sequencing of amplicons was performed at High Throughput Genomics Center (Seattle, WA, USA).

Phylogenetic analyses
For COI, P.patagonica s.l. sequences from Weis et al. (2014) (n=34 including five downloaded from NCBI) and Harder et al. (2016) (n=26) were added to the final data set. ITS sequences of specimens of both previous studies were generated and included into the ITS alignment, too. For both gene regions, sequences were edited with Geneious v. 8.1.3 (http://www.geneious.com, Kearse et al. 2012) and aligned in Geneious using MAFFT v. 1.3.3 Multiple Sequence Alignment (Katoh & Standley 2013) with default parameters as implemented in Geneious, with a gap opening penalty of 1.53 and offset value of 0.123. For COI, sequences were translated into amino acids using the invertebrate mitochondrial genetic code (translation table 5) to verify that all codons could be translated without stop codons. For ITS, a version of the alignment where ambiguously aligned regions were removed was produced with Gblocks v. 0.91b (Castresana 2000) using less stringent parameters (smaller blocks, gaps in final alignment allowed, less strict flanking positions) as has been done in Dietz et al. 2015b. For analyses when only unique copies were needed, sequences were collapsed into unique sequences ('haplotypes' for COI data) with the online tool FaBox v. 1.41 (Villesen 2007). For both, nuclear and mitochondrial data sets a maximum-likelihood (ML) analysis was performed with RAxML v. 8.2.4 (Stamatakis 2014) using the GTRCAT model of sequence evolution and branch support was assessed with 10000 rapid bootstrap replicates. In addition, for the mitochondrial data set a resolved ultrametric gene tree was calculated using BEAST v. 1.8.3 (Drummond et al. 2012) with the model specified by jModelTest v. 2.1.10 (Guindon & Gascuel 2003; Darriba et al. 2012). An XML file was created with BEAUti v. 1.8.3 (Drummond et al. 2012) with the following settings: HKY+G+I as substitution models and 80 x 10^6 as length of MCMC chain sampling every 1000th tree. Convergence of the likelihood and appropriate effective sampling size (ESS > 200) of parameter estimates were checked using TRACER v. 1.6 (Rambaut et al. 2014), and a consensus tree was calculated using TREEANNOTATOR v. 1.8.3 of the BEAST package. Furthermore, uncorrected pairwise distance matrixes were created using MEGA v. 7 (Tamura et al. 2011).

2.4 Species delimitation methods
For species delimitation analysis of the COI data set ABGD (Automatic Barcode Gap Discovery; Puillandre et al. 2012) was run to test for presence of distinct clades. As no clear barcode gap was found in the pairwise distance data, ABGD results varied drastically depending on single sequences and run parameters. Hence, for results presented default settings but Kimura-2-parameter (K2P) for distance were used. The same settings

were applied to the ITS alignment (including and excluding ambiguously aligned regions). Due to the smaller data set for the ITS alignment and the fact that informative alignment gaps cannot easily be interpreted as additional character in the tree-based delimitation methods, further species delimitation methods were only applied to the COI data set. The final mitochondrial COI ML tree was used to perform a Bayesian Poisson Tree Processes (bPTP) analysis using the web server (http://species.h-its.org/ptp; Zhang et al. 2013). Furthermore, a Generalized Mixed Yule Coalescent (GMYC) analysis based on the resolved ultrametric gene tree was conducted at the web server (http://species.h-its.org/gmyc; Fujisawa & Barraclough 2013) using the single-threshold method only (see Fujisawa & Barraclough 2013).

2.5 Molecular clock analysis
A calibrated molecular clock rate for sea spiders has not been reported in previous studies. However, in order to infer possible divergence date ranges for the different clades we applied a widely adopted COI molecular clock rate reported for insects: 1.15% permyr and lineage (Brower 1994). BEAST v. 1.8.2 was used to estimate divergence times using an HKY+I+G evolution model as well as an uncorrelated local clock model. Analyses were run for 10×10^6 generations sampling every 1000th tree. Convergence of parameter estimates and ESS control and subsequent steps were done as described above.

Data Processing Description

BCO-DMO Processing notes:
- added conventional header with dataset name, PI name, version date
- modified parameter names to conform with BCO-DMO naming conventions
- added links to NCBI accession pages
- changed commas in latitude and longitude to decimals
- replaced empty cells with nd (no data)

[table of contents | back to top]

Data Files

File
Domel_T1.csv (Comma Separated Values (.csv), 38.58 KB) MD5:b06b69e422995f6b9867f7afc82fd058 Primary data file for dataset ID 671948

[table of contents | back to top]

Related Publications

ARANGO, C. P., & BRENNEIS, G. (2013). **New species of Australian *Pseudopallene* (Pycnogonida: Callipallenidae) based on live colouration, morphology and DNA**. Zootaxa, 3616(5), 401–436. doi:10.11646/zootaxa.3616.5.1
Methods

Castresana, J. (2000). Selection of Conserved Blocks from Multiple Alignments for Their Use in Phylogenetic Analysis. Molecular Biology and Evolution, 17(4), 540–552. doi:10.1093/oxfordjournals.molbev.a026334
Methods

Dietz, L., Arango, C. P., Dömel, J. S., Halanych, K. M., Harder, A. M., Held, C., … Leese, F. (2015). Regional differentiation and extensive hybridization between mitochondrial clades of the Southern Ocean giant sea spider Colossendeis megalonyx. Royal Society Open Science, 2(7), 140424. doi:10.1098/rsos.140424
Methods

Drummond, A. J., Suchard, M. A., Xie, D., & Rambaut, A. (2012). Bayesian Phylogenetics with BEAUti and the BEAST 1.7. Molecular Biology and Evolution, 29(8), 1969–1973. doi:10.1093/molbev/mss075
Methods

Dömel, J. S., Melzer, R. R., Harder, A. M., Mahon, A. R., & Leese, F. (2017). Nuclear and Mitochondrial Gene Data Support Recent Radiation within the Sea Spider Species Complex Pallenopsis patagonica. Frontiers in

Ecology and Evolution, 4. doi:10.3389/fevo.2016.00139
Results

Folmer, O., Black, M., Hoeh, W., Lutz, R., and Vrijenhoek, R. (1994). DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Mol. Mar. Biol. Biotechnol. 3, 294–299
Methods

Fujisawa, T., & Barraclough, T. G. (2013). Delimiting Species Using Single-Locus Data and the Generalized Mixed Yule Coalescent Approach: A Revised Method and Evaluation on Simulated Data Sets. Systematic Biology, 62(5), 707–724. doi:10.1093/sysbio/syt033
Methods

Guindon, S., & Gascuel, O. (2003). A Simple, Fast, and Accurate Algorithm to Estimate Large Phylogenies by Maximum Likelihood. Systematic Biology, 52(5), 696–704. doi:10.1080/10635150390235520
Methods

Harder, A. M., Halanych, K. M., & Mahon, A. R. (2015). Diversity and distribution within the sea spider genus Pallenopsis (Chelicerata: Pycnogonida) in the Western Antarctic as revealed by mitochondrial DNA. Polar Biology, 39(4), 677–688. doi:10.1007/s00300-015-1823-8
Related Research

Katoh, K., & Standley, D. M. (2013). MAFFT Multiple Sequence Alignment Software Version 7: Improvements in Performance and Usability. Molecular Biology and Evolution, 30(4), 772–780. doi:10.1093/molbev/mst010
Methods

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., … Drummond, A. (2012). Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics, 28(12), 1647–1649. doi:10.1093/bioinformatics/bts199
Methods

Puillandre, N., Lambert, A., Brouillet, S., and Achaz, G. (2011). ABGD, Automatic Barcode Gap Discovery for primary species delimitation. Molecular Ecology, 21(8), 1864–1877. doi:10.1111/j.1365-294x.2011.05239.x
https://doi.org/10.1111/j.1365-294X.2011.05239.x
Methods

Stamatakis, A. (2014). RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics, 30(9), 1312–1313. doi:10.1093/bioinformatics/btu033
Methods

Sunnucks, P., & Hales, D. F. (1996). Numerous transposed sequences of mitochondrial cytochrome oxidase I-II in aphids of the genus Sitobion (Hemiptera: Aphididae). Molecular Biology and Evolution, 13(3), 510–524. doi:10.1093/oxfordjournals.molbev.a025612
Methods

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., & Kumar, S. (2011). MEGA5: Molecular Evolutionary Genetics Analysis Using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. Molecular Biology and Evolution, 28(10), 2731–2739. doi:10.1093/molbev/msr121
Methods

Villesen, P. (2007). FaBox: an online toolbox for fasta sequences. Molecular Ecology Notes, 7(6), 965–968. doi:10.1111/j.1471-8286.2007.01821.x
Methods

Weis, A., Meyer, R., Dietz, L., Dömel, J. S., Leese, F., & Melzer, R. R. (2014). Pallenopsis patagonica(Hoek, 1881) - a species complex revealed by morphology and DNA barcoding, with description of a new species ofPallenopsis Wilson, 1881. Zoological Journal of the Linnean Society, 170(1), 110–131. doi:10.1111/zoj.12097
Related Research

Weiss, M., & Leese, F. (2016). Widely distributed and regionally isolated! Drivers of genetic structure in Gammarus fossarum in a human-impacted landscape. BMC Evolutionary Biology, 16(1). doi:10.1186/s12862-016-0723-z
Methods

Zhang, J., Kapli, P., Pavlidis, P., & Stamatakis, A. (2013). A general species delimitation method with applications to phylogenetic placements. Bioinformatics, 29(22), 2869–2876. doi:10.1093/bioinformatics/btt499
Methods

Parameters

Parameter	Description	Units
species	taxonomic genus and species name	unitless
clade	clade identifier	unitless
Name	accession number or other identifier	unitless
lat	latitude; north is positive	decimal degrees
lon	longitude; east is positive	decimal degrees
depth	depth collected	meters
ZSM_voucher_number	ZSM voucher number	unitless
COI_GenBank_BOLD_number	COI - GenBank/ BOLD number	unitless
ITS_GenBank_BOLD_number	ITS - GenBank/ BOLD number	unitless
COI_NCBI_link	link to NCBI GenBank accession page	unitless
ITS_NCBI_link	link to NCBI GenBank accession page	unitless

Instruments

Dataset-specific Instrument Name	
Generic Instrument Name	Automated DNA Sequencer
Dataset-specific Description	Sequencing performed at High Throughput Genomics Center (Seattle, WA) and at GATC Biotech AG (Cologne, Germany)
Generic Instrument Description	General term for a laboratory instrument used for deciphering the order of bases in a strand of DNA. Sanger sequencers detect fluorescence from different dyes that are used to identify the A, C, G, and T extension reactions. Contemporary or Pyrosequencer methods are based on detecting the activity of DNA polymerase (a DNA synthesizing enzyme) with another chemoluminescent enzyme. Essentially, the method allows sequencing of a single strand of DNA by synthesizing the complementary strand along it, one base pair at a time, and detecting which base was actually added at each step.

Dataset-specific Instrument Name	
Generic Instrument Name	Thermal Cycler
Generic Instrument Description	A thermal cycler or "thermocycler" is a general term for a type of laboratory apparatus, commonly used for performing polymerase chain reaction (PCR), that is capable of repeatedly altering and maintaining specific temperatures for defined periods of time. The device has a thermal block with holes where tubes with the PCR reaction mixtures can be inserted. The cycler then raises and lowers the temperature of the block in discrete, pre-programmed steps. They can also be used to facilitate other temperature-sensitive reactions, including restriction enzyme digestion or rapid diagnostics. (adapted from http://serc.carleton.edu/microbelife/research_methods/genomics/pcr.html)

[table of contents | back to top]

Deployments

LMG1312

Website	https://www.bco-dmo.org/deployment/666516
Platform	ARSV Laurence M. Gould
Report	http://dmoserv3.bco-dmo.org/jg/serv/BCO-DMO/OA_Antarctic_organisms/727518.html0%7Bdir=dmoserv3.whoi.edu/jg/dir/BCO-DMO/OA_Antarctic_organisms/,info=dmoserv3.bco-dmo.org/jg/info/BCO-DMO/OA_Antarctic_organisms/mg_ca_ratios%7D
Start Date	2013-11-22
End Date	2013-12-20
Description	Benthic invertebrate studies

NBP1210

Website	https://www.bco-dmo.org/deployment/568987
Platform	RVIB Nathaniel B. Palmer
Report	http://dmoserv3.bco-dmo.org/jg/serv/BCO-DMO/OA_Antarctic_organisms/727518.html0%7Bdir=dmoserv3.whoi.edu/jg/dir/BCO-DMO/OA_Antarctic_organisms/,info=dmoserv3.bco-dmo.org/jg/info/BCO-DMO/OA_Antarctic_organisms/mg_ca_ratios%7D
Start Date	2013-01-06
End Date	2013-02-09
Description	Seaglider AUV-SG-503-2012 was recovered on this cruise.

[table of contents | back to top]

Project Information

Genetic connectivity and biogeographic patterns of Antarctic benthic invertebrates (Antarctic Inverts)

Coverage: Antarctica

Extracted from the NSF award abstract:

The research will explore the genetics, diversity, and biogeography of Antarctic marine benthic invertebrates, seeking to overturn the widely accepted suggestion that benthic fauna do not constitute a large, panmictic population. The investigators will sample adults and larvae from undersampled regions of West Antarctica that, combined with existing samples, will provide significant coverage of the western hemisphere of the Southern Ocean. The objectives are: 1) To assess the degree of genetic connectivity (or isolation) of benthic invertebrate species in the Western Antarctic using high-resolution genetic markers. 2) To begin exploring planktonic larvae spatial and bathymetric distributions for benthic shelf invertebrates in the Bellinghausen, Amundsen and Ross Seas. 3) To continue to develop a Marine Antarctic Genetic Inventory (MAGI) that relates larval and adult forms via DNA barcoding.

[ ]

Funding

Funding Source	Award
NSF Office of Polar Programs (formerly NSF PLR) (NSF OPP)	PLR-1043745
NSF Office of Polar Programs (formerly NSF PLR) (NSF OPP)	PLR-1043670

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