A taxonomic revision of the Caridean shrimp, Pasiphaea tarda (Krøyer, 1845)

Thesis for the degree of Master of Science
Marine Biology

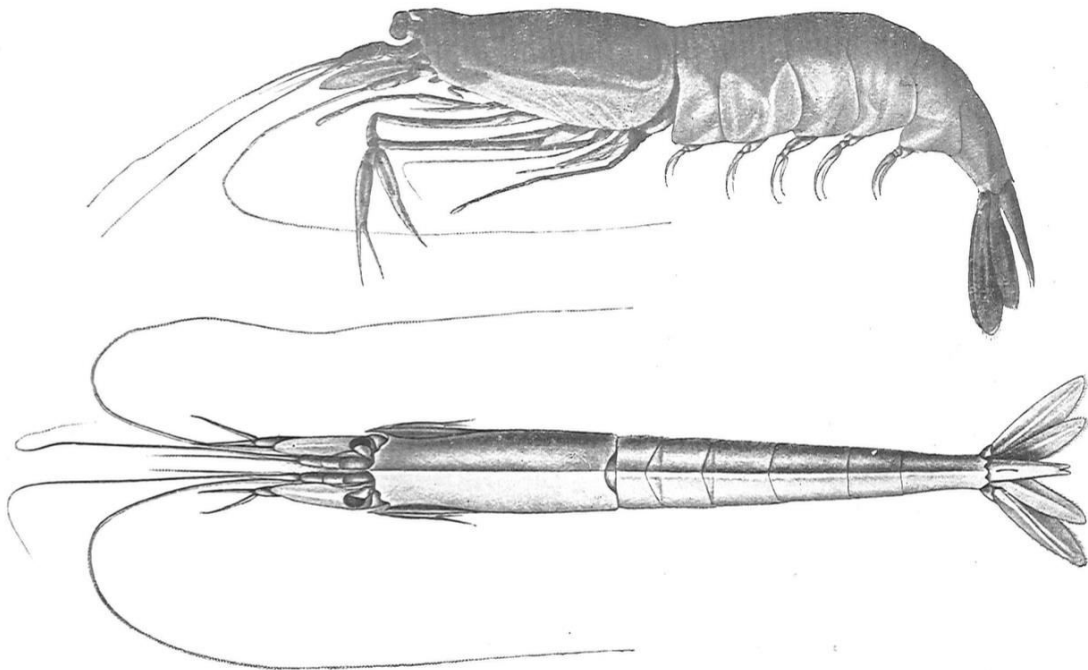by Fredrik Egeland Bjørnestad

Image taken from (Sund, 1913)

Department of Biology
University of Bergen

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Abstract

The taxonomic status of the Caridean shrimp, *Pasiphaea tarda (Krøyer, 1845),* has been a matter of debate throughout the years, and various authors have argued whether or not *P. princeps* and *P. principalis* should be synonymized with *P. tarda*. A preliminary phylogenetic NJ tree placing Atlantic sequences in a group distinct from Pacific sequences has added to the dispute. DNA were extracted from Pacific and Atlantic specimens and sequenced for the mitochondrial cytochrome c oxidase subunit 1 gene (COI). The sequences were supplemented by Pasiphaeid sequences downloaded from GenBank and aligned. A phylogenetic tree was created using Maximum likelihood and the topology was confirmed with Bayesian inference. The tree indicated divergence between a monophyletic Atlantic lineage, and two distinct lineages in the Pacific. Nonetheless, K2P distances were in accordance with the populations belonging to the same species. ANOVAs conducted on morphological data found no significant ($p > 0.05$) differences within the Atlantic Ocean. Substantiated by K2P distances, this indicates a homogenous population of *P. tarda* within the Atlantic. ANOVAs comparing the Pacific and Atlantic population found significant differences ($p < 0.05$) between the two populations. The divergent morphology and the degree of genetic divergence between the Atlantic and Pacific populations indicate limited gene flow and that *P. tarda* is polytypic. The time of divergence was estimated to 1 mya, and was calculated using Bayesian inference and a mutation rate of 0.014/Myr. Failing to identify any distinguishing morphological characters of taxonomic importance between *P. princeps* and *P. tarda* prompts a synonymization of the two taxa.

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1.0 Introduction

The crimson pasiphaeid, *Pasiphaea tarda*, is a relatively large species of shrimp (infraorder: *Caridea*), belonging to the family *Pasiphaeidae.* The genus *Pasiphaea* consists of seventy accepted species (Fransen, 2015), with three species known to inhabit Norwegian waters (Artsdatabanken, 2016). Its size and distribution is inadequately documented in the literature, and is usually based on a small sample size. However, the data gathered for this thesis documents that *P. tarda* is able to grow to lengths (measured from rostrum to telson) of at least 200mm. The data also shows that the species has a documented presence in major parts of the North-Atlantic, from as far north as Baffin Bay in the west and northern Norway in the East, and as far south as the Bay of Biscay. *P. tarda* is also documented to be present in the northeastern parts of the Pacific, off the coast of Canada and Alaska. The shrimp lives in the pelagic and benthic environment, and it is documented in the literature (Butler, 1980, Sund, 1913, Kemp, 1910), supplemented by data collected for this thesis, to be present at depths of 150 to at least 2100 meters. As is common for meso- and bathypelagic species (Johnsen, 2005) it is red in color(Butler, 1980).

The systematic status of *P. tarda* has been subject of several controversies since its description by the Danish zoologist Henrik Krøyer in 1845. There are disagreements among various authors about the distinction between the species *P. tarda, P. princeps* and *P. principalis* (now synonymized with *P. tarda*), and to some extent *P. multidentata*, and whether or not they are the same species, two, three or four distinct species. Smith (1882) describes the new species *P. princeps* in the West Atlantic Ocean, however, it is debatable how it differs from *P. tarda (Sivertsen and Holthuis, 1956)*. Kemp (1910) refers to specimens corresponding to *P. tarda*'s morphology by the name *P. princeps,* claiming he has documented the first presence of *P. princeps* in the East Atlantic. In the same text he also uses the name *P. tarda* when referring to specimens corresponding to *P. multidentata*'s morphology. Sund (1913) describes the new species *P. principalis,* claiming that the material determined by Kemp (1910) to be *P. princeps* is

actually this new species, *P. principalis* (now synonymized with *P. tarda*), and not *P. princeps* as Kemp (1910) claimed. Sund is a proponent of the 4 species supposition, differentiating between the species *P. multidentata, P. tarda, P. princeps* and *P. principalis.* Sivertsen and Holthuis (1956) states that the opposing viewpoint, that all four species should be synonymized, is held by J. Stephenson in an article from 1912. Sivertsen and Holthuis (1956) argue that *P. tarda* and *P. principalis* should be synonymized, which they later have been. Examining the arguments made by Smith (1882), Smith (1886) and Sund (1913), as well as material identified by S. I. Smith, the authors also conclude that there are no differences of taxonomic importance between *P. tarda* and *P. princeps* and that the two species should be regarded as one. Iwasaki (1990) agrees with Sivertsen and Holthuis (1956) in that *P. tarda* and *P. principalis* should be synonymized, but believes that *P. tarda* is distinct from *P. princeps*, listing 6 defining characters that can be used to distinguish the two species (discussed later).

In the present study specimens of *P. tarda* were collected in the Sognefjord by researches at the University of Bergen during surveys as part of a Sognefjord research project in 2011, 2012 and 2013. The Sognefjord project is a cooperative undertaking between the Institute of Marine Research and the University of Bergen, set to map the biodiversity of the Sognefjord. Morphological examinations of the specimens collected revealed that several of the specimens collected only had one spike on the basis of the second pereiopod (Prof. Henrik Glenner, personal communication, 2015). The original description of *P. tarda* reports the species having 3 spikes on the basis joint (Krøyer, 1845), and in literature known to the researchers the reported number of spikes on the basis of the second pereiopod was 2-7 (Prof. Henrik Glenner, personal communication, 2015). As part of a preliminary study, DNA from four specimens of *P. tarda* collected in the Sognefjord was extracted, and the mitochondrial cytochrome c oxidase 1 gene (COI) was sequenced. Sequences reported to belong to *P. tarda* from the North East Pacific (n=4) and Rosemary Bank northwest of Scotland in the Atlantic (n=3), as well as sequences of *P. multidentata* (n=8) and *P. sivado*

(n=6) were downloaded from GenBank and aligned. Using the alignment a
Neighbor Joining tree (NJ-tree) was created and can be viewed in figure 1.1
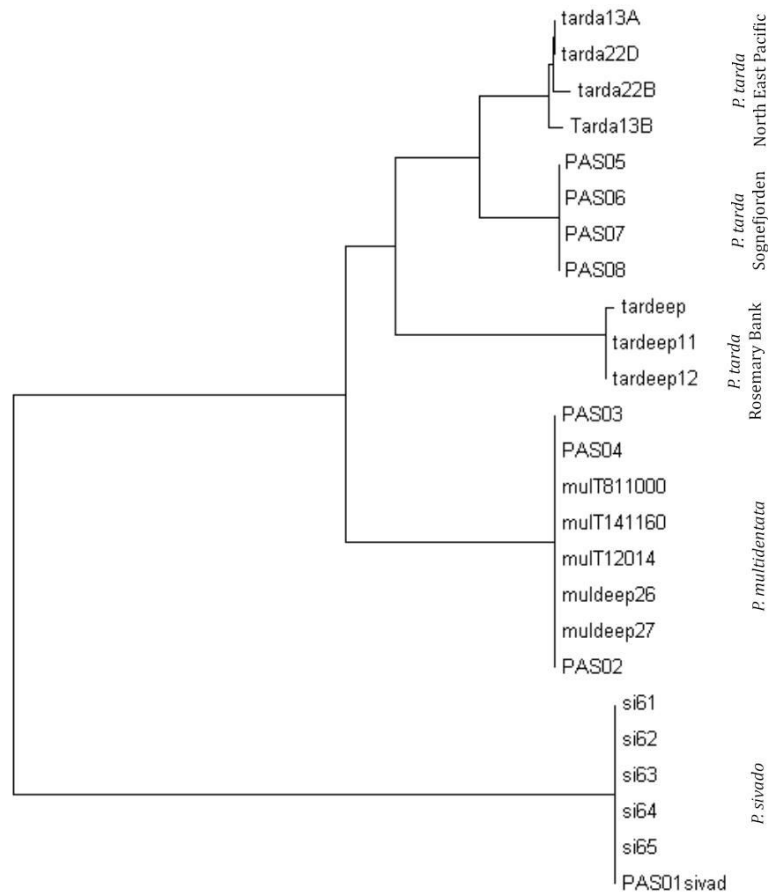
Figure 1.1. Preliminary NJ-tree indicating the phylogenetic relationship between the five groups; *P. sivado, P. multidentata*, *P. tarda* (Rosemary Bank group), *P. tarda* (Sognefjorden group) and *P. tarda* (North Pacific Group).

The preliminary NJ-tree seen in figure 1.1 indicates that all five groups are genetically distinct, with the Pacific and the Sognefjord populations of *P. tarda* having the most similar genotype, implying a relatively close phylogenetic relationship. A distance analysis was also conducted, producing a p-distance of 3,2% between the two groups, providing further evidence describing the degree of divergence between the Atlantic and Pacific populations of *P. tarda*. The Pacific and Sognefjorden population showed a p-distance of 7% and 6,7% to the *P. tarda* specimens from Rosemary Bank, respectively. However, the Sognefjord sequences were genetically identical to COI sequences extracted from specimens collected at the Mid-Atlantic Ridge, not included in the phylogenetic tree in figure

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1.1 (Rees, 2015, unpublished work).These findings indicates that *P. tarda* quite possibly is a species complex, consisting of three distinct taxa; one in the Pacific, and two in the Atlantic. The data also suggested that the two taxa living in the Atlantic have an overlapping habitat, with one genotype having a documented presence in both Sognefjorden and at the Mid-Atlantic Ridge, and the other taxon having a documented presence somewhere in the middle of these two locations, at Rosemary Bank northwest of Scotland.

The disagreements among authors in the pre-existing literature on whether or not *P. princeps* and *P. tarda* are distinct species or if they should be synonymized, augmented by the data from the preliminary study conducted as part of the Sognefjord project, suggesting that *P. tarda* is a species complex possibly consisting of three distinct species, prompts a taxonomic revision of the taxon. This study aims at reviewing the taxonomic status of the crimson pasiphaeid, *Pasiphaea tarda*. A morphological study will be conducted on specimens collected at various geographical locations. The data gathered will be analyzed statistically, potentially uncovering significant morphological differences of taxonomic importance between populations. This study also aims at supplementing the pre-existing literature, giving a broader description of interspecific character variation, with emphasis on characters often listed in the literature as species defining characters within the *Pasiphaea* genus. The collected specimens will be fixed in a preservative medium, which keeps the genetic material viable for DNA sequencing. DNA will be extracted and the mitochondrial cytochrome c oxidase 1 (COI) gene will be sequenced due to its general usefulness for taxonomic classification at taxonomic levels from phylum to species for most metazoans, including crustaceans (Costa et al., 2007, Schander and Wilassen, 2005, Hebert et al., 2003). Supplemented by sequences downloaded from GenBank, the genetic information stored within the COI gene will be used to reconstruct the phylogeny of the P*asiphaea* genus in a phylogram. The genetic variation within and between populations will be analyzed, providing taxonomic information that, together with the morphological data and the reconstructed phylogeny, can be used to determine if *P. tarda* is a species complex, to what degree the different populations are related, and if any genetic

divergence can be coupled to any marked morphological trait. The information might also finally resolve the disputed taxonomic relationship between *P. tarda* and *P. princeps*, although it is doubtful that any genetic material is attainable from the latter species due to what is presumed about the availability, age and preservative used for conserving specimens of *P. princeps*. The taxonomic relation between the two species will, therefore, have to be resolved on the basis of morphological character differences. The phylogenetic relationship between the two Atlantic populations will be further investigated, with the relative large p-distance (6,7%) between the two populations hinting at an incorrect determination of species in the case of the three sequences collected at Rosemary Bank. The relatively high genetic p-distance (3,2%) between the Atlantic and the Pacific population hints at these populations having limited interpopulation gene flow. It is therefore likely to assume that the divergence between the Atlantic and Pacific population originated due to the formation of a separating barrier limiting larval dispersal and gene flow. Using Bayesian inference with a fixed clock model and an empirical mutation rate, an estimate of when the separation between the groups happened will be conducted, and an effort will be made to see if the estimated time since divergence can be correlated to any major geographic and/or oceanographic event.

2.0 Materials and Methods

2.1 Specimen Collection

The specimens being studied in this thesis were acquired by loan from the institutions listed in table 2.1 below. One trip was also made to The University of Copenhagen, where a morphological inspection was done on site, including the inspection of the type material of *Pasiphaea tarda*. A full list of the specimens studied in this thesis can be found in Appendix 1.

Table 2.1. Summary of the institutions providing material for the thesis, including the institutions location and the number of specimens provided. Specimens of *P. princeps* are listed in parentheses.

Institution	Location	Number of Samples
University Museum of Bergen	Bergen, Norway	103
National Museum of Scotland	Edinburgh, Scotland	15
Royal British Columbia Museum	Victoria, BC, Canada	34
Canadian Museum of Nature	Ottawa, ON, Canada	6
Smithsonian/National Museum of Natural History (P. princeps)	Washington DC, USA	(5)
The Biodiversity Institute of Ontario, University of Guelph	Guelph, ON, Canada	2
The University of Copenhagen Zoological Museum	Copenhagen, Denmark	28
Total		188 (193)

The specimens listed in table 2.1 above have been collected from a vast area, including both the Atlantic and Pacific Oceans, with collection dates ranging from the mid 1800's until present. An illustration of where the specimens have been collected is illustrated in figure 2.1 on the next page. A comprehensive list containing information about when and where the specimens were collected can be found in Appendix 1.
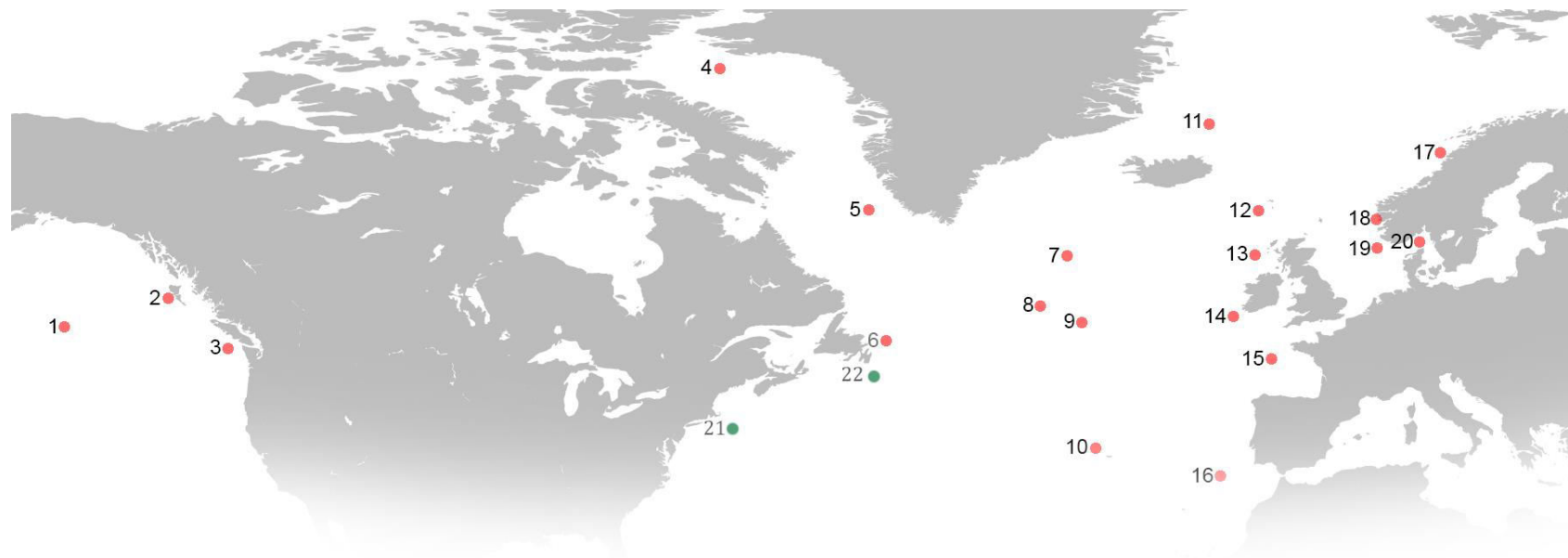
	Location	n Specimen(s)		Location	n Specimen(s)
1	Ocean Station Papa	4	11	Western Norwegian Sea	4
2	Graham Island Area	13	12	Faroe Islands	15
3	Vancouver Island Area	19	13	Scotland Area	2
4	Baffin Bay Area	1	14	West of Ireland	7
5	Greenland	6	15	Bay of Biscay	2
6	New Foundland Area	3	16	Portugal Area	1
7	Mid Atlantic Ridge Area 1	1	17	Nordland Area	8
8	Mid Atlantic Ridge Area 2	12	18	Sogn og Fjordane & Horaland Area	30
9	Mid Atlantic Ridge Area 3	17	19	Nordsjøen	4
10	Azores	3	20	Skagerak Area	22

	Location	n Specimens
21	NE Coast U.S.A	3
22	SE Coast Canada	2

Total = 174   **Total = 5**

Figure 2.1. Map indicating the areas from which the specimens studied in this thesis were collected. Locations where specimens of *P. tarda* have been collected are marked with a red dot, and locations where specimens of *P. princeps* have been collected are marked with a green dot. The number of specimens collected at each site is listed in the tables in the lower left corner. Some specimens are not accounted for in this figure due to lacking information about where they were collected.

2.2 DNA extraction, amplification and sequencing

2.2.1 Extraction and purification

Genetic material was collected by sampling a 2-5 mm piece of tissue from the pleopod of each individual specimen. Of the specimens successfully sequenced all were preserved in 96% EtOH, except for two samples (sample: 010-00247-011 and 291015-1) being preserved in 70% EtOH. For an entire list of specimens successfully sequenced see Appendix 1.1. Extraction of the genetic material was performed by using the Qiagen DNeasy Blood & Tissue Kit following the Purification of Total DNA from Animal Tissues protocol (QIAGEN, 2006).

Before commencing the extraction of DNA, the tissue was diluted by transferring it to individual Eppendorf® tubes containing 500μL of ddH$_2$O for approximately 2.5 hours. The ddH$_2$O was then removed, and the samples were allowed to dry for 5 minutes. 180μL ATL buffer, and 20μL of Proteinase K was then added to each sample, and the tubes were subsequently vortexed and centrifuged for 3 seconds. The samples were now ready to be lysed, and was placed in block incubators at 56°C for 3-24 hours. When the tissue was fully lysed the Eppendorf® tubes were vortexed for 15 seconds, breaking down any remaining undissolved tissue.

To purify the DNA, 200μL of Buffer AL and 200μL of EtOH were added to each Eppendorf® tube and vortexed for 3 seconds. The whole content (600μL) was then transferred to the DNeasy Mini Spin Columns and centrifuged for 1 minute at 8000 rpm. The collection tube and its content were discarded, and the spin column was placed in a new collection tube. 500μL of Buffer AW1 was added to each column and subsequently centrifuged for 1 minute at 8000 rpm. The collection tube and its content were discarded, and the spin column was placed in a new collection tube. 500μL of Buffer AW2 was added to each column and subsequently centrifuged for 4 minutes at 13000 rpm. The collection tube and its content were discarded, and the spin column was placed in Eppendorf® tubes. 200μL buffer AE was added to the spin column before being centrifuged at 8000 rpm for 1 minute. An additional 200μL of AE was added to the same spin column before another round of centrifugation at 8000 rpm for 1 minute. The genetic material was now purified, and the extracts were stored in a fridge at 4°C.

2.2.2 Amplification and PCR product quantification

Amplification of an approximately 710-bp stretch of the mitochondrial cytochrome c oxidase subunit 1 gene (COI) was done by utilizing the Polymerase Chain Reaction (Mullis et al., 1986). Table 2.2 specifies the reactants and the respective volumes of each reactant needed to amplify 1µL of DNA extract. Depending on number of extracts being amplified, the volumes in table 2.2 were simply multiplied, being careful to make sufficiently enough for approximately 5 additional extracts, accounting for a positive and negative control, as well as accounting for any inaccuracies. The mixture of reactants, referred to as a master mix, was mixed ahead of the addition of DNA extracts. 1µL DNA extract, described in section 2.2.1, was individually put into PCR tubes, and 24µL of the master mix was then added to each tube. The PCR tubes, each containing 25µL of reactants, were then placed in a thermal cycler running the program "Barcoding" (Appendix 2).

Table 2.2. Reactants and volumes needed to prepared one DNA extract for the Polymerase Chain Reaction. The Takara Taq utilized is the Qiagen HotStar+ Takara Taq, and the Primers are the standard Folmer primers(Folmer et al., 1994).

Reactants	Volume (µL)
H_2O	18.2
10x Buffer	2.5
dNTPs	1.2
Primer 1 (LCO 1490)	1
Primer 2 (HCO 2198)	1
Takara Taq	0.15
Total	**24**

To quantify the amount of DNA product obtained from the Polymerase Chain Reaction, the product was run through an electrophoresis gel consisting of 1% agarose, 99% TAE-buffer, and depending on the amount of gel used (table 2.3), a set amount of DNA stain (GelRed™). The liquid gel was poured into a casting block, wells were added, and the gel was let to solidify for approximately 20 minutes. The gel was then submerged in TAE-buffer. Each well was then loaded with a mixture of 1µL loading dye (Ficoll™) and 4µL PCR product. As a reference to the DNA fragments, one of the wells was loaded with 4µL of the ladder (FastRuler™). An electric potential of 90V was administered across the gel,

and the PCR products were let to wander the gel for approximately 30 minutes. The gel was then analyzed in a Syngene UV Cabinet (Syngene, Cambridge, UK), and band quantification was determined using the programs GeneSnap (GeneSnap, version 7.01, 2007) and GeneTools (GeneTools, version 4.00, 2008).

Table 2.3. Amount of GelRed™ added to the electrophoresis gel depending on the amount of gel being used.

Gel (mL)	GelRed™ (μL)
30	1
50	3
100	6

2.2.3 Final purification and sequencing

The PCR products were purified by removing leftover primers and dNTPs by the use of the enzymes Exonuclease I (EXO I) and Shrimp Alkaline Phosphatase (SAP). A master mix of reactants was prepared according to table 2.4. The reactant volumes listed in table 2.4 were multiplied by the numbers of PCR products being sequenced. To prevent any enzyme degradation, all preparations of the master mix were conducted on a bed of ice. 2μL of the master mix, as well as 8μL of PCR product, were individually added to PCR tubes, centrifuged for 3 seconds, and placed in a thermal cycler running the program "EXOSAP" (Appendix 2).

Table 2.4. Reactants used to remove leftover primers and dNTPs in the PCR products before sequencing.

Reactant	Volume (μL)
EXO 1	0.1
SAP	1.0
dH$_2$O	0.9
Total	**2.0**

Preparing the PCR products for sequencing was done according to the BigDye® version 3.1 sequencing protocol (Uib.no, 2016). This is a 10μL reaction and the reactants and their respective volumes can be seen in table 2.5. The reactants were individually pipetted into PCR tubes and placed in a thermal cycler running the program "SEQ" (Appendix 2). Upon completion 10μL dH$_2$O was added to each PCR tube, and

subsequently delivered to the sequencing facility at the University of Bergen. Here an automated Sanger DNA Sequencing procedure is performed using a capillary-based Applied Bio system 3730XL Analyzer. The finished sequences were then uploaded to the sequencings facility's server as .AB1 files.

Table 2.5. Reactants used in a BigDye version 3.1 sequencing protocol. The amount of DNA/PCR product (0.5µL - 4µL) is determined during the band quantification in section 2.2.2 by using the programs GeneSnap and GeneTools (Syngene, Cambridge, UK).

Reactants (µL)	Volume (µL)
DNA/PCR Product	x
dH_2O	7 - x
LCO 1490 Primer	1
Sequencing buffer	1
BigDye®	1
Total	**10**

2.3 Morphological analysis

The morphological analysis of the specimens was performed by visual inspection under a stereomicroscope. The examination was aided by the use of forceps and needles to manipulate the posture and position of the specimens. To prevent the specimens from drying out and degrading during examination, the specimens were put in a petri dish filled with a solvent corresponding to the specimen fixative. The fixative was either 96% EtOH, 70% EtOH or Isopropanol (Appendix 1.3).

To ensure that the collection of data corresponded to the species of interest, *Pasiphaea tarda*, each specimen examined was first identified with the use of an identification key supplemented by defining characters described in pre-existing literature. Christiansen (1972) offered a key sufficient to separate the Norwegian species *P. tarda, P. sivado* and *P. multidentata* and Crosnier and Forest (1973) described the differences between *P. tarda* and *P. ecarina.* These characters are summarized in the identification key on the next page.

1. Dorsal keel present on the abdominal segments
    a. Yes…………………………….……..2
    b. No   *P. ecarina*
2. Split Telson
    a. Yes………………………………...3
    b. No   *P. sivado*
3. Number of spikes on basis of the 2nd pereiopod
    a. 7-12   *P. multidentata*
    b. 1-5   *P. tarda*

A number of measurements were taken from each specimen for later use in the comparative analysis of the specimens and their corresponding population. The type of measurements taken, the tools used to take the measurements, along with an explanatory description of the measurements are summarized in table 2.6. A complete table of the specimens examined and their corresponding character parameters are listed in Appendix 1.2.

Table 2.6. A summary of the characters measured, the tools used to take the measurements and an explanatory description of how the measurements were taken.

Character	Measuring tool	Description
Total length (cm)	Measuring tape	Measured dorsally from tip of telson to tip of rostrum
Length of Carapace (mm)	Digital caliper	See figure 2.2.
Spikes on Basis of 2nd Pereiopods	Stereo Microscope	-
Spikes on Ischium 2nd Pereiopods	Stereo Microscope	-
Lateral Length of Scaphocerite	Digital caliper	Not including the anterior spike. See figure 2.3.
Width of Scaphocerite	Digital caliper	Widest part of scaphocerite. See figure 2.3.
Rostrum	Stereo Microscope	Assigned to one out of 4 categories. See figure 2.4.
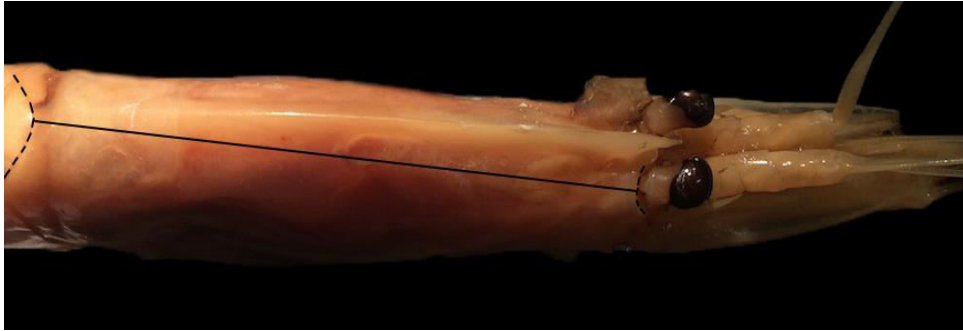
Figure 2.2. The length measurement of the carapace was taken slightly diagonally on the dorsal side, starting at the posterior margin of the carapace until reaching the eye socket.

Figure 2.3. Description of where the measurements of the scaphocerite were taken. The x-axis indicated the path of the length measurement. The y-axis indicates the path of the width measurement taken at the widest part of the scaphocerite.
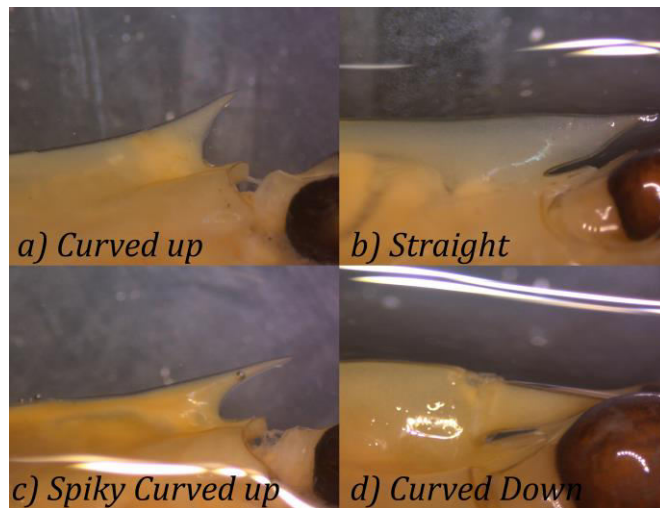
Figure 2.4. During examination the specimens were assigned to either one of four rostrum phenotype categories; a) curved up b) straight c) spiky curved up d) curved down

2.4 Data analysis

2.4.1 Sequence adjustments and alignment

The sequences prepared in section 2.2.3 were downloaded from the University of Bergen's Sequencing facility's servers as .AB1 files. The .AB1 trace files were inspected and roughly edited in the DNA trace file software 4Peaks (4Peaks, version 1.8, 2015). The sequence ends were trimmed and ambiguity codes were edited into the sequences in compliance with IUPAC notation where necessary. The sequences were then exported as .FastA files. Additional sequences were downloaded from GenBank (Appendix 1.2) and all sequences were compiled into a single .FastA file consisting of 72 individual sequences.

The DNA sequences were subjected to multiple sequence alignment using the inbuilt Clustal Omega software (Clustal Omega, version 1.2, 2015) in SeaView (SeaView, version 4.5.4, 2015). The ends of the sequences were again trimmed to limit the variation of length between the sequences, resulting in an alignment 620pb long. Determining the reading frame of the alignment and the detection of any possible stop codons within the sequences was done by amino acid translation in the sequence analysis software MEGA (MEGA, version 6.06, 2015).

2.4.1 Distance Estimation

Using the built in Neighbor-Joining method (NJ) in SeaView (SeaView, version 4.5.4, 2015) with 500 bootstraps, a preliminary phylogenetic tree was generated. The clades suggested by the NJ-tree gave a rough estimation of how to group the sequences in a group distance analysis. Within group mean distances, and between group mean distances were calculated according to the Kimura 2-parameter model (K2P) (Kimura, 1980) in MEGA (MEGA, version 6.06, 2015). Parameters were set the to 2000 bootstraps, using a nucleotide substitution model including both transitions and transversions.

2.4.2 Maximum Likelihood Tree

A maximum likelihood tree (ML) was generated by using the inbuilt ML tree function in MEGA (MEGA, version 6.06, 2015). The parameters were set to 2000 bootstraps, using a nucleotide substitution model with a tamura 3-parameter model + gamma with 5 categories and invariable sites (T92 + G + I). The ML heuristic model was set to nearest-neighbor-interchange (NNI), and the initial tree for ML was set to NJ/BIONJ. The branch swap filter was set to very strong.

The model used to generate the ML tree, T92 + G + I, was selected by using the built in "Find best DNA model (ML)" function in MEGA (MEGA, version 6.06, 2015). The resulting models and their respective parameters can be seen in Appendix 3.

2.4.3 Bayesian tree with time of divergence

The .FastA file alignment generated in section 2.4.1 was converted to a .NEXUS file format by utilizing the conversion option in SeaView (SeaView, version 4.5.4, 2015). The .NEXUS file was then opened in the Bayesian evolutionary analysis software, BEAUTi (BEAUTi, version 1.8.2, 2015). The substitution model was set to GTR, base frequencies were set to Estimated, heterogeneity model was set to Gamma and Invariable Sites, and Gamma categories was set to 5. A strict clock model was chosen, with a mutation rate of 0.014/Myr (Knowlton and Weight, 1998). The Marcov Chain Monte Carlo (MCMC) parameter was set to a chain length of 20000000, with parameter loggings every 1000. An .xml file was generated, and subsequently opened in BEAST (BEAST, version 1.8.2, 2015) where a tree file was generated. The tree file was opened in TreeAnnotator (TreeAnnotator, version 1.8.2, 2015) where burn-in was set to 2000 (10%) and the posterior probability was set to 0.99. A tree-file was then generated and subsequently opened in FigTree (FigTree, version 1.4.2, 2015) to produce a Bayesian tree showing the posterior probability values for each node and estimates of node divergences.

Evaluating the validity of using a singe evolutionary rate along all branches was performed by the "likelihood ratio test" (LRT) (Lemey and Posada, 2009). The tree generated in section 2.4.2 was exported as a .nwk file and the content of this file was

copied and pasted into the NEXUS-file generated earlier in this section. By utilizing the program PAUP* (PAUP*, version 4.0, 2002) likelihood scores were generated for both the rooted constrained tree and a de-rooted unconstrained version of the tree. Both scores were noted, and the "likelihood ratio" was tested utilizing the program Modeltest (Modeltest, version 3.7, 2005).

2.4.4 Statistical analysis

Statistical analysis of the morphological data was executed in the statistics software RStudio (RStudio, version 0.98, 2013). For count data an ANOVA test with a general linearized model (GLM) and quasipoisson distribution (accounting for overdispersion) was used. If the F-test showed a significant effect, a post-hoc Tukey's HSD test for individual means was performed. When analyzing the relationship between size and rostrum type, the categorical response variable, "rostrum type", was converted into numerical proportions [0-1] (0=Spiky curved up, 0,33=curved up, 0,67=straight, and 1=curved down), and an ANOVA test with a quasibinomial distribution was used. For continuous data an ANOVA test with a linear model (LM) was used. When the ANOVA showed a significant effect from the predictor, a Tukey's HSD test for individual means was performed. The normality and homogeneity of variance was tested for all models, and the alpha level was set to 0.05. To control for the effect of size, the parameter "carapace length" was added as a covariate to all models, controlling that the observed differences between groups are not caused by differences in the size distribution within the data sets. Furthermore, the interaction between the predictor variables was also analyzed, and if no statistically significant interaction were detected, interaction was not included in the final model.

Some of the data lacked a sufficient sample size to perform ANOVAs between the three groups *P. tarda* (Pacific population), *P. tarda* (Atlantic population) and *P. princeps.* This data was displayed by the use of histograms to give information about the range of variability within character traits, the most common phenotype for the individual groups, and possibly hint at possible character differences distinguishing the groups from each other.

3.0 Results

3.1 Genetic analysis

3.1.1 Distance analysis

A distance analysis was conducted to assess the mean K2P distance within the groups defined in table 3.1. The analysis was conducted utilizing an alignment of the COI gene compromising 72 individual sequences belonging to 8 distinct species. The analysis produced mean K2P distances summarized in table 3.1. The lowest mean K2P distance within a group, 0,04%, was found in the Atlantic group of *P. tarda,* indicating that this group is genetically homogenous. However, the largest mean K2P distance within a group, 1,49%, was found in the Pacific group of the same species, *P. tarda*. The phylogeny of this group (see the phylogenetic tree in section 3.1.2) exposed that the Pacific group contained one sequence (specimen ID: 010-00247-011) highly divergent compared to the other sequences in the same group. The analysis just described was therefore performed once more, now with the divergent sequence removed from the Pacific *P. tarda* group*.* This reduced distance within the Pacific group from 1,49% to 0,92%, exposing that much of the genetic diversity within this group could be ascribed to this single specimen. Nevertheless, the genetic diversity was still much higher within the Pacific group, than in the Atlantic group.

Table 3.1. Mean distance within groups estimates based on the Kimura 2-parameter model (K2P) between the groups specified in the table with 2000 bootstraps. The number of sequences within each group is specified in the left column. Both the mean K2P distance within group and standard errors are given.

Species	p-distances	S.E.
P. sivado (n=13)	0,15%	0,07%
P. planidorsalis (n=2)	0,32%	0,22%
P. telacantha (=3)	0,43%	0,23%
P. hoplocerca (n=1)	–	–
P. pacifica (n=5)	0,48%	0,17%
P. multidentata (n=18)	0,14%	0,06%
P. sp. (Rosemary Bank) (n=3)	0,12%	0,12%
P. tarda (Atlantic) (n=17)	0,04%	0,02%
P. tarda (Pacific) (n=6)	0,92%	0,24%
P. tarda (excluding Pacific_010-00247-011) (n=5)	–	–
P. tarda (n=28)	1,36%	0,25%

A distance analysis between groups was also conducted utilizing the same alignment as in the analysis of genetic distance within groups. This analysis produced mean K2P distances summarized in table 3.2 and 3.3. This showed a maximum mean K2P distance of 31,64% within the *Pasiphaea* genus. The Pacific and Atlantic populations of *P. tarda* showed a distance of 3,11% (see in table 3.2). Furthermore, this distance analysis was repeated with the divergent sequence removed from the Pacific group. This increased the K2P distance between the main Pacific and Atlantic -group to 3,19%, and showed a distance of 2,69% and 2,64% from the divergent sequence to the Atlantic and Pacific groups, respectively. The taxonomically unidentified group from the Rosemary Bank northwest of Scotland showed the least distance to the species *P. tarda* (Atlantic group), with a K2P distance of 7,29%. The K2P distance between *P. multidentata* and *P. tarda* was 8,48%.

Table 3.2. Mean distance between groups estimates based on the Kimura 2-parameter model (K2P) between the Atlantic and Pacific populations of *P. tarda* with 2000 bootstraps. The number of sequences within each group is specified in the left column. The distances are given as percent and are written in black. The standard error is written in blue.

Groups	*P. tarda* (Atlantic)	*P. tarda* (Pacific)
P. tarda (Atlantic) (n=21)		0,61%
P. tarda (Pacific) (n=6)	3,11%	

Table 3.3. Mean distance between groups estimates based on the Kimura 2-parameter model (K2P) between the groups specified in the matrix with 2000 bootstraps. The number of sequences within each group is specified in the left column. The distances are given as percent and are written in black. The standard error is written in blue.

Groups	P. sivado	P. planidorsalis	P. telacantha	P. hoplocerca	P. pacifica	P. multidentata	P. sp. (Rosemary_Bank)	P. tarda (Atlantic)	P. tarda (Pacific excluding 010-00247-011)	P. tarda (Pacific 010-00247-011)
P. sivado (n=13)		2,04%	1,76%	3,03%	2,53%	2,44%	2,58%	2,54%	2,63%	2,64%
P. planidorsalis (n=2)	21,66%		1,73%	2,99%	2,63%	2,47%	2,61%	2,40%	2,55%	2,58%
P. telacantha (=3)	17,70%	16,51%		2,94%	2,53%	2,30%	2,47%	2,41%	2,46%	2,51%
P. hoplocerca (n=1)	30,58%	31,64%	31,52%		2,43%	2,30%	2,41%	2,39%	2,31%	2,52%
P. pacifica (n=5)	28,59%	30,28%	29,20%	23,98%		2,04%	2,13%	2,14%	2,17%	2,26%
P. multidentata (n=18)	26,22%	26,97%	24,57%	22,13%	19,53%		1,48%	1,25%	1,29%	1,15%
P. sp. (Rosemary Bank)) (n=3)	25,73%	26,51%	24,80%	21,65%	20,58%	10,17%		1,19%	1,29%	1,16%
P. tarda (Atlantic) (n=21)	28,05%	26,74%	26,85%	22,87%	20,58%	8,48%	7,29%		0,65%	0,71%
P. tarda (Pacific excluding 010-00247-011) (n=5)	29,11%	28,11%	27,44%	22,58%	21,50%	8,92%	8,29%	3,19%		0,66%
P. tarda ((Pacific) ID number: 010-00247-011) (n=1)	23,99%	24,36%	24,44%	21,07%	20,02%	6,20%	6,23%	2,69%	2,64%	

3.1.2 Phylogenetic tree
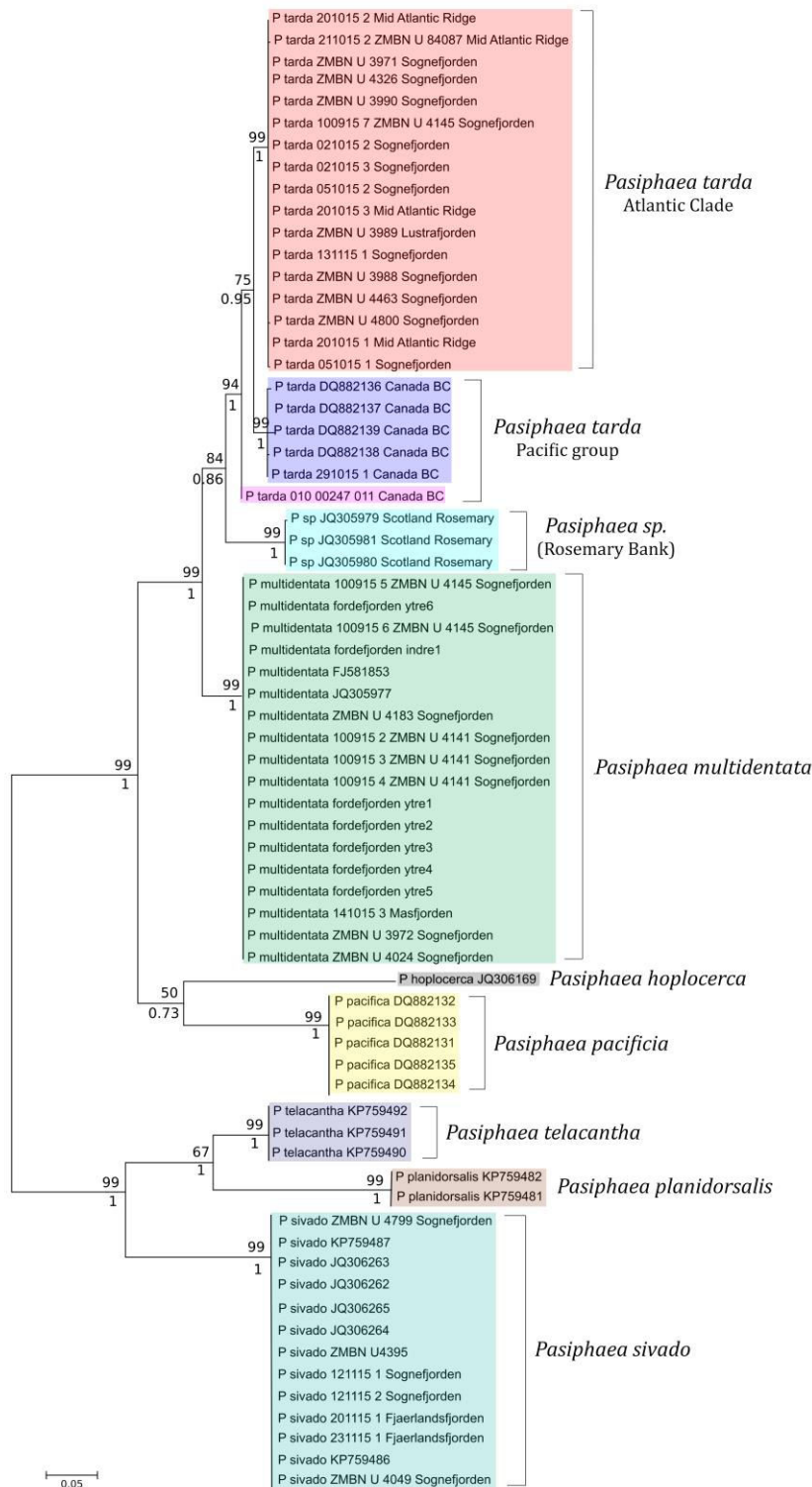
Figure 3.1. Phylogenetic tree of the *Pasiphaea* genus (including 8 of 70 accepted species) created using Maximum likelihood with 2000 bootstraps under the best fitting model, T92+G+I. The topology was confirmed using Bayesian inference under the model GTR+G+I run with 20000000 generations, with the first 2000000 discarded as burn-in. Bootstrap values are indicated above the nodes, and the posterior probabilities are indicated below the nodes.

The phylogeny of the *Pasiphaea* genus is visualized in the phylogenetic tree in figure 3.1. The tree is based on the 620 bp long alignment of the mtDNA COI gene. The alignment consists of 72 sequences, belonging to 7 identified species, and 1 unidentified species. The unidentified species group consists of three sequences taken from the Rosemary Bank area northwest of Scotland. This group shows the closest phylogenetic relationship with *P. multidentata* and *P. tarda*. *P. tarda* is divided into 3 groups, with one Atlantic group, and two Pacific groups, with the phylogeny indicating that the larger Pacific group (n=5) is more closely related to the Atlantic group than the Pacific group consisting of one single divergent sequence (ID number: 010-00247-011).

3.1.3 Estimating time of divergence

The cladogram in figure 3.2 was created using Bayesian inference utilizing the same dataset used to create the phylogram in figure 3.1. Estimates of time since most recent common ancestor for the major nodes are shown at all major nodes and are given in myr. These time estimates are based on a strict clock approach with a rate of 0.014/Myr (Knowlton and Weight, 1998). With these assumptions the tree infers that the unidentified species (*P. sp.*) collected at the Rosemary Bank northwest of Scotland had it's most recent common ancestor with the *P. tarda* clade about 2.93 mya. Furthermore, the tree indicates that the Atlantic population separated from the Pacific population (the large monophyletic group) about 0.98 mya. The divergent Pacific sequence (ID number: 010-00247-011) is indicated to have had a most recent common ancestor with the two other *P. tarda* groups about 1.3 mya, about 0.35 myr before the split up between the Pacific and Atlantic populations.
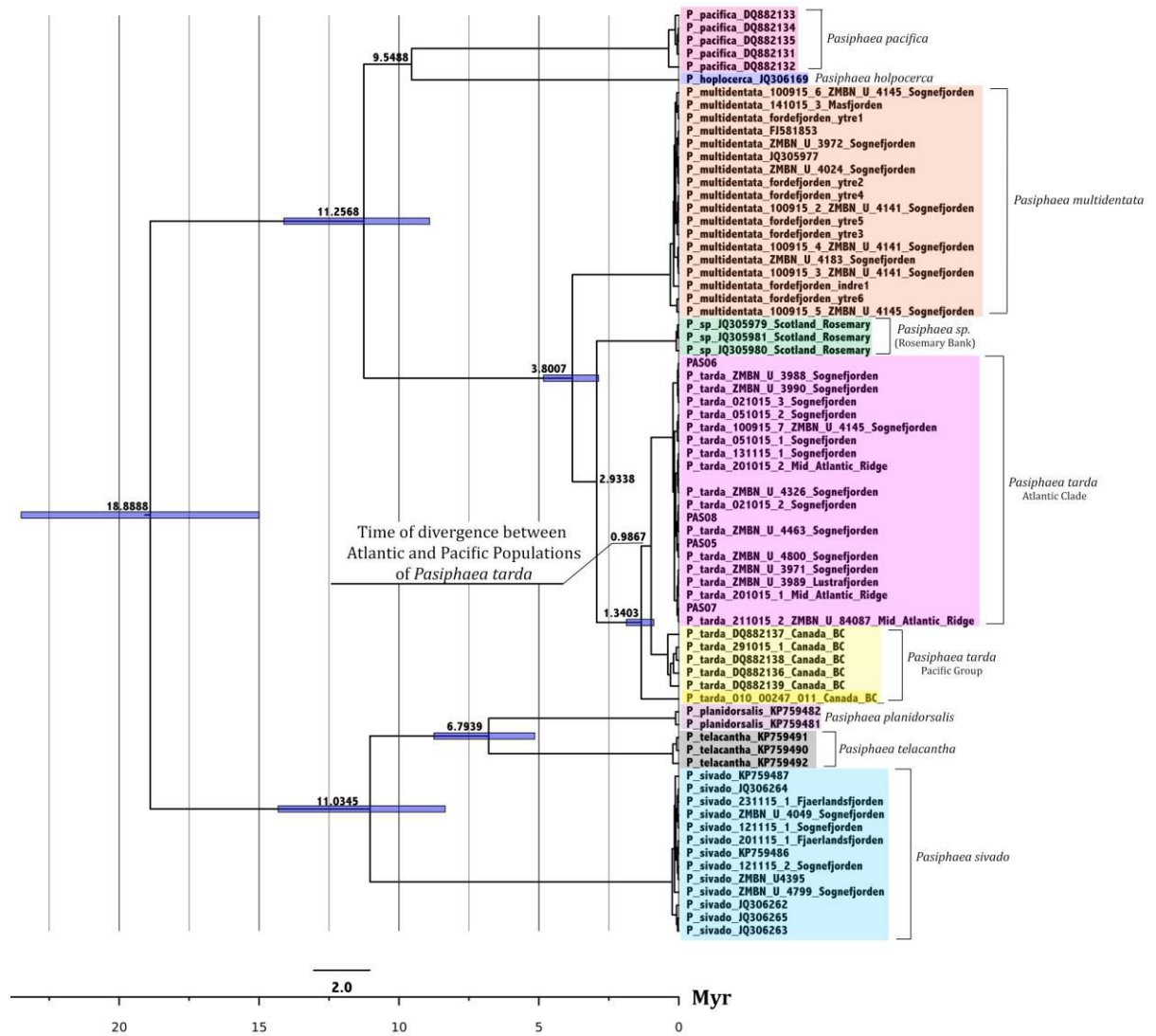
Figure 3.2. Cladogram created using Bayesian inference for the Genus *Pasiphaea* (including 8 species) created using a strict clock approach under the model GTR+G+I run with 20000000 generations, with the first 2000000 discarded as burn-in. Estimates of time since most recent common ancestor for the major nodes are given in Myr, and the 95% highest posterior density (HPD) for the nodes are shown as blue horizontal bars.

3.2 Morphological analysis

3.2.1 Statistical analysis on morphological character traits between groups

Statistical analysis of the morphological data was executed using ANOVA conducted in the statistical software RStudio (RStudio, version 0.98, 2013). The data was first ordered into 4 distinct groups; One Pacific group (east_pacific), and three Atlantic groups based on geographic origin (west_atlantic, mid_atlantic and east_atlantic). The statistical analysis executed did not find any statistical difference (p>0.05)) in the number of spikes on basis of the second pereiopod between any of the four groups. Furthermore, four additional parameters were tested: a) Spikes on ischium of the 2nd Pereiopod, b) Scaphocerite length and carapace length ratio, c) Total length and carapace length ratio, and d) Width of scaphocerite (scaphocerite width/length). A significant difference (p<0.05) was found when comparing the parameters b), c) and d) between the Pacific and Atlantic groups, however, no such difference was found when comparing the Atlantic groups to each other, with minor exceptions. A small (0.12), but significant (p=0.022) difference in the "Total length and carapace length ratio" parameter was detected when comparing the "east_atlantic" and "mid_atlantic" groups. When comparing parameter a) there was only a significant difference (p=0.015) in the number of spikes on the ischium of the 2nd pereiopod between the "east_pacific" group and the "mid_atalantic" group. The same statistical tests mentioned above were also performed with the data from the three Atlantic groups (west_atlantic, mid_atlantic and east_atlantic) merged into single Atlantic group (atlantic). There was no statistical significant difference (p>0.05) detected in the number of spikes on basis of the 2nd pereiopod, or in the number of spikes on the ischium of the 2nd pereiopod between the two populations. The results of the ANOVA for parameters b), c) and d) are summarized in table 3.4, and the differences between the Atlantic and Pacific populations are illustrated with boxplots in figure 3.4.

Table 3.4. Summary of the statistically significant (p<0.05) ANOVA tests performed comparing the parameter b), c) and d) between the Pacific and Atlantic population of *P. tarda* . The relative differences between the populations are also given, as well as the corresponding p-value for each test.

Parameter	Pacific population	Difference	Atlantic population	Relative difference (Pacific/Atlantic)	P value
b) Scaphocerite length and carapace length ratio (carapace/scaphocerite length)	2,061	-0,146	1,915	-7,60%	$4,17 \times 10^{-07}$
c) Total length and carapace length ratio (total length/carapace length)	3,207	0,039	3,246	+1,2%	0.0408
d) Width of scaphocerite (width/length)	0,304	-0,048	0,256	-18,80%	2×10^{-16}
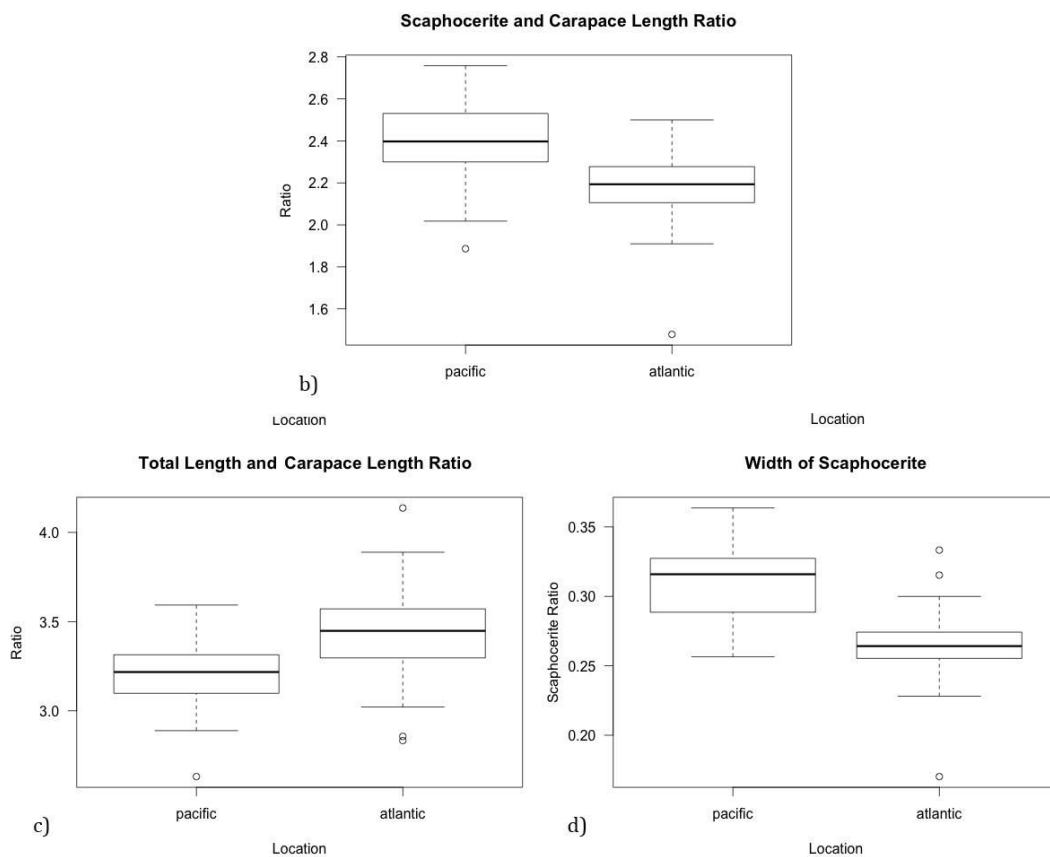
Figure 3.4. Boxplots showing the difference between the Pacific and Atlantic population with regards to the parameters b) Scaphocerite length and carapace length ratio, c) Total length and carapace length ratio, and d) Width of scaphocerite (scaphocerite width/length). The corresponding p-values are listed in table 3.4, except for parameter a) Spikes on ischium of 2nd pereiopod which a non-significant p-value of 0.498.

3.2.2 Phenotypes related to size

Selecting the carapace length as an indicator of size the data showed that there were significant differences ($p<0.05$) in the size distributions between the "mid_atlantic" and "east_pacific" groups, the "east_atlantic" and "east_pacific" groups, and between the "east_atlantic" and "west_atlantic" groups when comparing the data as four distinct groups. When combining the Atlantic populations into one group the ANOVA showed a difference of 7,4mm in size between the Pacific and Atlantic group ($p=6,72 \times 10^{-05}$), with the Pacific group mean size being 37,7mm, and the Atlantic mean size being 30,3mm. This makes the specimens from the Pacific 24,4% larger than the Atlantic specimens.

Correlation tests were performed to see if the any character traits where correlated with the size of the specimens. The length of the carapace was selected as the best indicator of size, and this parameter was tested against the "Scaphocerite width", "Spikes on basis of 2nd pereiopod" and the "Spikes on ischium of 2nd Pereiopod" parameter. A positive correlation of 0,237, 0,340 and 0,131 was found, respectively. The plots in figure 3.5 illustrate these relationships, showing that the "Scaphocerite width", "Spikes on basis of 2nd pereiopod" and the "Spikes on ischium of 2nd Pereiopod" parameter values are increasing with increasing size of the specimen.
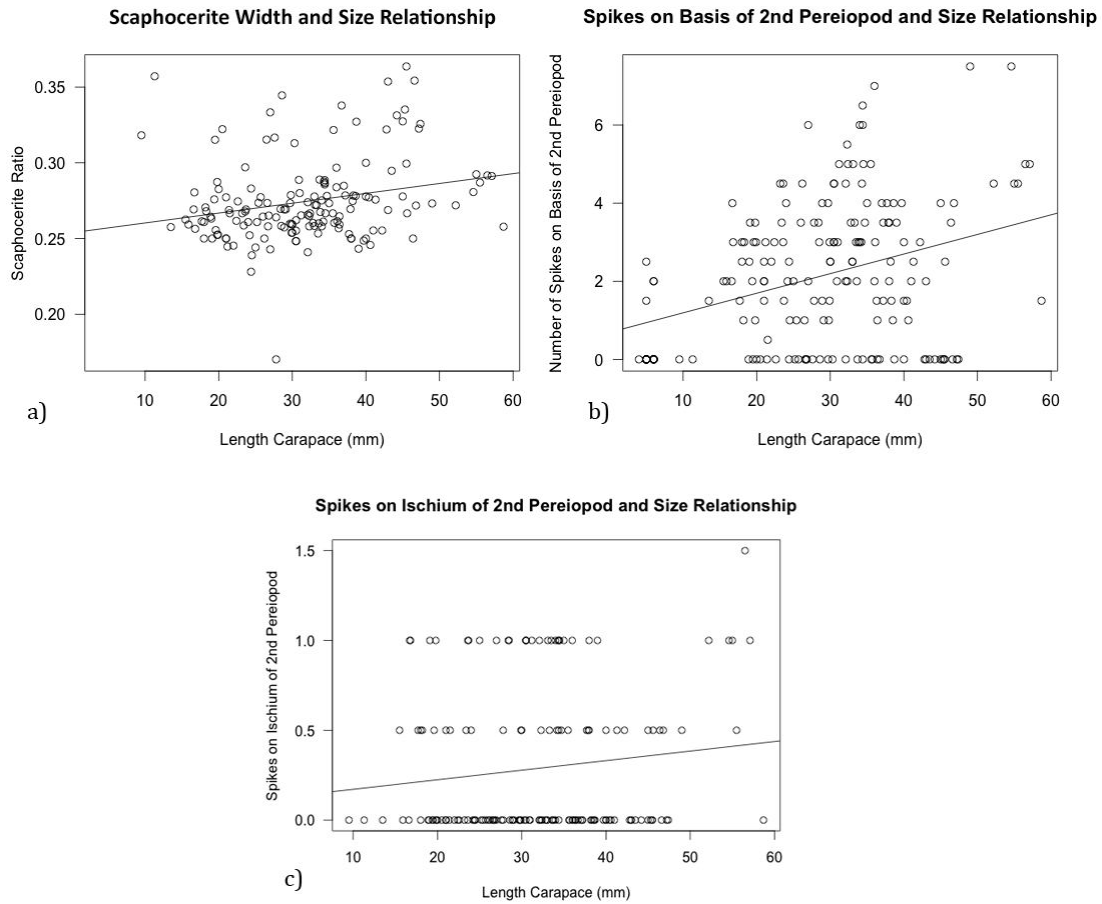
Figure 3.5. Scatterplots with regression lines illustrating the positive correlation between size and a) Scaphocerite width, b) Spikes on basis of 2nd pereiopod and c) Spikes on ischium of 2nd pereiopod.

The categorical data describing the rostrum shapes of the specimens examined was converted into numerical proportions [0-1] (0=Spiky curved up, 0,33=curved up, 0,67=straight, and 1=curved down). To check if there are changes in this phenotype related to size, the converted data was plotted against the length of the carapace, with the latter parameter functioning as an indicator of general size. A probability curve was drawn on the plot and can be seen in figure 3.6. The figure asserts that certain rostrum phenotypes are more common at certain sizes, implying a morphological evolvement of this character correlated with an increase of size.
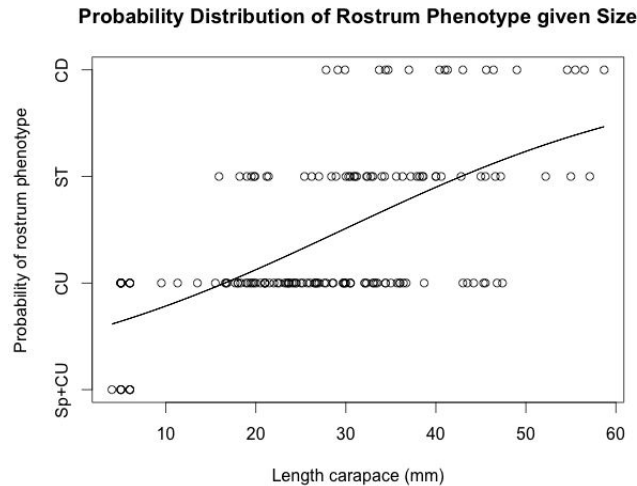
Figure 3.6. Scatterplot with probability curve of rostrum type given size . The categorical data for rostrum type was converted into numerical proportions ranging from 0-1, seen on the y-axis. The abbreviations Sp+CU, CU, ST, and CD are used for "spiky curved up", "curved up", "straight" and "curved down", respectively.

3.2.3 Frequency distribution of spikes on pereiopod segments

The number of spikes on the basis, ischium and merus segments of the 2nd pereiopod, and the number of spikes on merus of the 1st pereiopod was recorded for the three groups: *P. tarda* (Atlantic population), *P. tarda* (Pacific population) and *P. princeps*. The frequency distributions are shown in figure 3.7. For both *P. tarda* groups the number of spikes on the merus segments (both 1st and 2nd pereiopod) was only recorded for specimens affiliated with genetic data confirming their taxonomic determination. Figure 3.7 a) indicates a similar frequency distribution for all three groups, with 0 spikes being the most common phenotype. However, the data indicates the Pacific population of *P. tarda* having the possibility of a higher amount of spikes on the basis segment, with a maximum of 9 spikes compared to 7 for the Atlantic population and 2 for *P. princeps*. Figure 3.7 b) indicates a similar frequency distribution for all three groups, with 0 being the most common phenotype. We also see a similar pattern as in figure 3.7 a), with the Pacific population of *P. tarda* having a higher range of spikes on the ischium segment of the 2nd pereiopod compared to the other groups. The lacking documentation of certain phenotypes in the *P. princeps* group can possibly be ascribed to a low sample size in this group. Figure 3.7 c) shows no clear differences between the three groups, possibly due to a low

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sample size in all groups. However, the figure indicates a great variation within this character trait, with overlap between all groups. The last figure (figure 3.7 d)) also has a low sample size, making it unreasonable to draw any major conclusions. There is nonetheless an overlap in the morphological data for the Atlantic group of *P. tarda* and *P. princeps*, with the Pacific group of *P. tarda* showing a higher amount of spikes on this appendage compared to the other two groups.
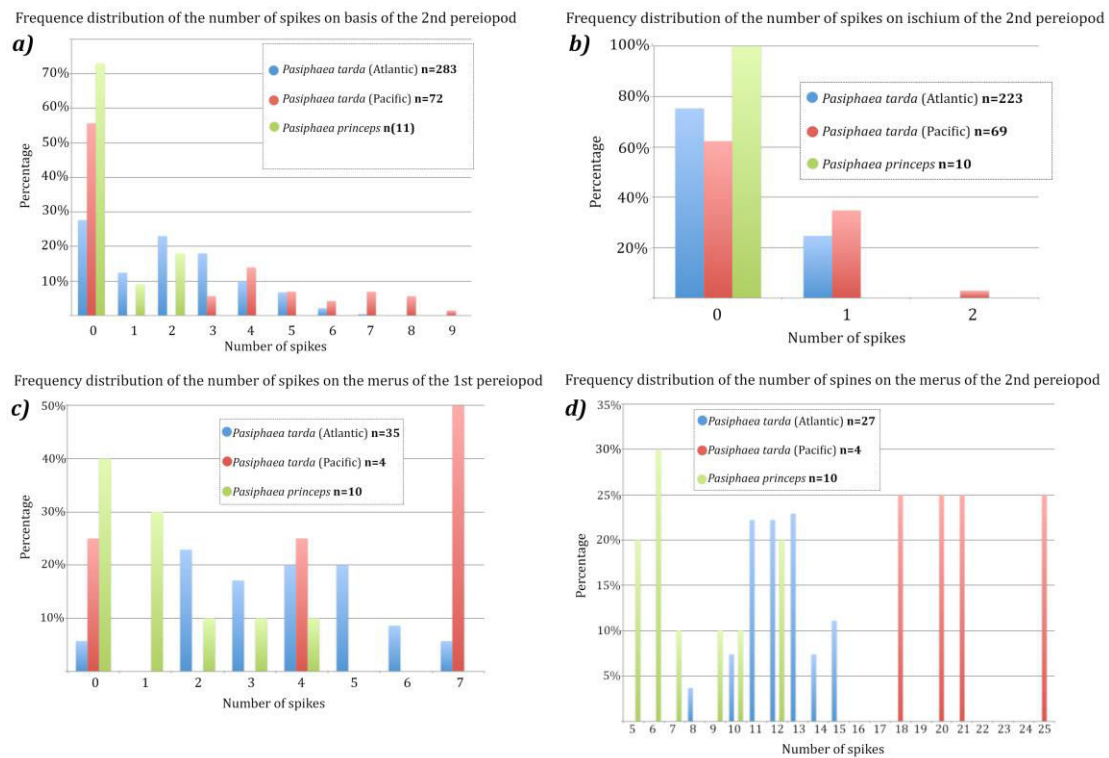
Figure 3.7. Histograms showing the frequency distribution of a) number of spikes on the basis of the 2nd pereiopod, b) number of spikes on ischium of the 2nd pereiopod, c) number of spikes on the merus of the 1st pereiopod and d) number of spikes on the merus of the 2nd pereiopod. Each histogram gives the frequency distribution for the three groups: *P. tarda* (Atlantic population), *P. tarda* (Pacific population) and *P. princeps*, with the color blue, red and green indicating the species, respectively. The sample size for each figure is also given and corresponds to the number of segments examined, not the number specimens examined. An estimate of the number of specimens examined for each group can be obtained by dividing the sample size in half.

4.0 Discussion – A Brief Overview

Genetic data belonging to eight of the seventy accepted species classified under the *Pasiphaea* genus was used to reconstruct the genus phylogeny. The reconstructed phylogeny, supplemented by K2P distances, provides a good estimate of the relatedness between the species within the genus, limited to the species with available sequences. Furthermore, complementary statistical analysis of the morphological data strongly suggest that *P. tarda* consists of at least two geographically segregated populations; one in the Atlantic Ocean, and at least one in the Pacific Ocean. Utilizing an empirical mutation rate, the data suggests that the two populations separated about 0.98 million years ago, with the Pacific population being the parent population. However, the relatively small genetic and morphological differences between the two populations suggest it is most appropriate to regard the two populations belonging to one polytypic taxon. The statistical analysis of the morphologic data also revealed an apparent evolvement of morphology related to size, with certain phenotypes being more prevalent at certain size intervals. The data collected for this study also supplements the pre-existing literature and expands the knowledge about the variation within character traits as well as providing a wider understanding of what the most common phenotypes for *P. tarda* are.

Sequences belonging to specimens collected from Rosemary Bank northwest of Scotland were previously determined to belong to the *P. tarda* taxon. The reconstructed phylogeny, augmented by K2P distances, suggests that the sequences belong to a species closely related to *P. tarda* with a mean K2P distance relatively similar to *P. multidentata*'s mean K2P distance from *P. tarda*.

Both the original description and type specimen of *P. princeps* were examined, and its morphology was compared to the known characteristics of *P. tarda.* The character parameters examined was found to be within the known range present in *P. tarda*. Parallel to previous authors, this study fails to find any characters suitable to distinguish the two species from each other. The few characters

provided in pre-existing literature to be apposite for this purpose are also generally disputed by the data gathered for this study. Having a similar morphology, as well as inhabiting the same habitat in the same oceans, suggests that *P. tarda* and *P. princeps* are the same species. A synonymization of *P. princeps* with *P. tarda* is therefore recommended.

4.1 Discussion of methodology

The title of this thesis is "A taxonomic revision of the Caridean shrimp, *Pasiphaea tarda*". However, there are no established methodology or definition describing how to undertake such a revision (Maxted, 1992). What methods to employ, the size of the data set, and the geographical areas being sampled are largely determined by what issues are being addressed, the pre-existing knowledge about the taxon and the availability of materials and data. The estimated duration of the project and the available founding are also determining factors that have to be taken into account. The scope of this thesis was largely a result of issues and findings revealed by the preliminary study part of the Sognefjord project mention in the introduction. Gene sequences from the Sognefjord, the Mid Atlantic Ridge and off the coast of British Columbia indicates that the Atlantic population of *P. tarda* is genetically distinct from the Pacific population of *P. tarda*. Some of the researcher also believes that a previously unknown phenotype of *P. tarda* has been discovered in the Sognefjord, however, this phenotype was genetically identical to the other available sequences from the Atlantic (Rees, 2015). This thesis wanted to answer whether or not the genetic differences between the Pacific and Atlantic populations can be reflected in their morphology, the degree of divergence between the populations and if the Atlantic meta-population is genetically and morphological homogenous, or if it consists of distinct sub-populations. To address these issues materials will have to be gathered from a large geographical area, and both morphological and molecular analysis will be employed on the material gathered. Morphological and molecular analysis have distinct advantages in a phylogenetic reconstruction, and applying both methods often produces results with the highest degree of explanatory power (Hills, 1987).

4.1.1 Material collection

One topic being addressed was whether or not the Atlantic population of *P. tarda* is morphologically and genetically homogeneous, and if the Atlantic population is genetically and/or morphologically distinct from the Pacific population. To ensure that the data obtained by further analyses is capable to answer such a question, material will have to be gathered from a large geographical area covering most of the Atlantic and Pacific Ocean. However, this was not feasible due to the limited availability of specimens and the time consuming process of tracking down, borrowing and obtaining specimens from other institutions. Nevertheless, within the scope of the thesis it was important to obtain material collected from the largest area possible. The material collected was limited to the North Atlantic and North East Pacific Oceans. The material with an origin in the Atlantic Ocean did, nonetheless, cover large parts of the North Atlantic Ocean, including samples from the East Atlantic, The Mid Atlantic Ridge and the West Atlantic. To ensure that the morphological and molecular analysis have the power to either corroborate or contradict the results from the analyses conducted it was ensured that either sequences or specimens with DNA viable for sequencing was obtained from all major geographic areas sampled. This decision is further substantiated by molecular analyses superior ability to discriminate between cryptic species, which might be the case for *P. tarda* (PACKER et al., 2009). The geographic overlap of the genetic and morphological data can be viewed in figure 4.2.1.

4.1.2 Choosing genetic marker

The genetic marker utilized in the molecular analyses of *P. tarda* was chosen to be the mitochondrial cytochrome c oxidase (COI) gene. Favoring the COI gene was based on both the inherent properties of the gene, as well as adventitious properties associated with the gene. The Consortium for the Barcode of Life (CBOL) is an international initiative devoted to developing DNA barcodes as an international standard for the identification of biological species (Barcodeoflife.org, 2016). The COI gene is the standard barcode gene affiliated

with CBOL due to its usefulness for taxonomic classification at taxonomic levels from phylum to species for most metazoans (Krishnamurthy and A., 2011, Schander and Wilassen, 2005, Hebert et al., 2003, Blaxter, 2003). The ability to resolve phylogenies in the specific case of decapoda is also demonstrated at the genus and species level (da Silva et al., 2011, Schubart, 2009), making the COI gene applicable for the analyses conducted as part of this thesis. Furthermore, assorted COI sequences belonging to members of the *Pasiphaea* genus are accessible at online databases such as GenBank, which is beneficial when reconstructing the phylogeny of the genus. The article by da Silva et al. (2011) provides range values of mean K2P distances within species, within genus and within family based on data obtained from 101 species belonging to 11 families within the Decapoda order, with the study using the COI gene as its sole genetic marker. Utilizing sequences derived from the COI gene in distance analyses would allow for direct comparison with the mean K2P distances provided by da Silva et al. (2011), making the COI gene a fitting candidate as the genetic marker used in this thesis.

4.1.3 Choosing characters for ANOVA

Precursory examination of specimens of *P. tarda* revealed no pronounced morphological peculiarities distinct for any of the geographic locations sampled. Selecting what character parameters to examine and record for any subsequent analysis were therefore influenced by the pre-existing literature and the featured characters documented or declared to be distinguishing characters for *P. tarda*, and to some extent *P. princeps* (Iwasaki, 1990, Sivertsen and Holthuis, 1956, Sund, 1913, Kemp, 1910, Smith, 1886, Smith, 1882, Krøyer, 1845). Considerations were also made to choose characters that were relatively easy to measure in a consistent and precise manner, taking into account the varying size, age and varying condition of the various specimens. One measurement in particular can arguably be criticized for failing to meet these criterions, and results based on this parameter are therefore emphasized to a minimal degree. The measurement in question is the total length of the specimens, being measured from the tip of the rostrum to the tip of telson along the dorsal side. The total length measurement noticeably varies depending on the posture of the

specimen measured, with lower values being recorded if the specimen is fully stretched out compared to when the pleon (abdomen) is in a curled up position. The specimens acquired for this thesis were either preserved in EtOH or isopropanol. Fixation in these mediums at the concentrations used results in relatively rigid and non-flexible specimens (King and Porter, 2003). Stretching out or positioning the specimens in the same posture was therefore not practicable when conducting this measurement, making this measurement prone to inaccuracies. Sund (1913) criticized the accuracy of this measurement for the same reason just presented. Nonetheless, despite its deficiencies, the measurement was used in in an analysis comparing the "total length/carapace length" ratio between the Atlantic and Pacific population of *P. tarda* revealing an apparent significant difference between the two populations (p = 0.0408). Most specimens were in approximately the same posture so it is plausible that the inaccuracies associated with this measurement have a minimal effect on the results from the subsequent analysis. However, a statistical assessment of the impact of the inaccuracies was not conducted and the results from the analysis based on the "total length/carapace length" should only be regarded as an indication of an actual difference between the groups. Furthermore, the power of some of the statistical analyses conducted are low due to the small sample size in some of the groups (Cohen, 1992), possibly leading to type II errors. Sample sizes for the groups analyzed are listed in figure 4.2.1.

4.2 Main results

4.2.1 The North Atlantic population of *P. tarda*

Prior to the statistical analyses, the morphological data from the Atlantic Ocean was ordered into three groups based on geographical origin and correspondingly named "West Atlantic", "Mid Atlantic" and "East Atlantic (seen in figure 4.2.1). Statistical analyses were conducted on the data to see if any difference could be detected between the groups with regards to the following parameters: a) Spikes on ischium of the 2nd Pereiopod, b) Scaphocerite length and carapace length ratio, c) Total length and carapace length ratio, d) Width of scaphocerite (scaphocerite width/length) and e) Number of spikes on basis of the second pereiopod. No statistically significant (p>0.05) difference was found between any of the groups, with one minor exception. A small (0.12), but significant (p=0.022) difference in the "Total length and carapace length ratio" parameter was detected when comparing the "East Atlantic" and "Mid Atlantic" groups. However, this minor difference between the groups can possibly be attributed to the inaccuracy of the "total length" measurement discussed in section 4.1.3. Nevertheless, the result generally gave evidence suggesting no morphological difference between the geographical areas investigated within the Atlantic Ocean. All the results from the analyses of the morphological data are presented in section 3.2.

The phylogenetic tree presented in figure 3.1 grouped the sequences from the "Mid Atlantic" group (area 7, 8 and 9, n=4) together with sequences from the "East Atlantic" group (area 18, n=13) in a monophyletic clade. The bootstrap value and posterior probability for this clade was 99 and 1, respectively. The mean K2P distance within this monophyletic group was only 0.04% (S.E. 0.02%). da Silva et al. (2011) used COI sequences from 101 species belonging to 11 families within the Decapoda order to produce mean K2P distances within species, genus and family. The mean K2P distance within species ranged from 0.285% to 1.375%. Comparing these values with the mean K2P distance within the group composed of sequences from area 7, 8, 9 and 18 (figure 4.2.1), it is

reasonable to conclude that despite being separated by a distance of approximately 2000km there are apparently no isolating barriers separating the Mid Atlantic Ridge and the Sognefjord, and the sequences from these locations all belong to the same species. No sequences were obtained from the northwest Atlantic Ocean, but COI sequences from this area are present on the boldsystems.org database. Although not available for the public to download, the webpage allows for uploading of sequences for identification, as well as generating a NJ tree composed of the uploaded sequence and similar sequences from the "Species Level Barcode Records" (Boldsystems.org, 2016). A NJ tree was constructed (seen in figure A4.1 Appendix 4), and COI sequences from the northwest Atlantic Ocean (area 6, figure 4.2.1) were placed in the same monophyletic group as sequences from the Sognefjord. This demonstrates that specimen found in the "East Atlantic", the "Mid Atlantic" and the "East Atlantic" groups are genetically the same.

The results from the morphological and genetic analyses discussed above provide compelling evidence demonstrating the presence of *P. tarda* in major parts of the North Atlantic Ocean. The results also demonstrate that *P. tarda* has limited genetic and morphological variation over a relatively large geographic area of distribution. This also hints at an apparent lack of isolating barriers in the North Atlantic Ocean for *P. tarda* as a species, and that *P. tarda* has a high dispersal potential (Palumbi, 2003). This knowledge coupled with the "Competitive Exclusion Principle" (Hardin, 1960) makes the possibility of a sympatric cryptic species resembling *P. tarda* inhabiting the North Atlantic Ocean unlikely, contrary to what the initial hypothesis suggested.
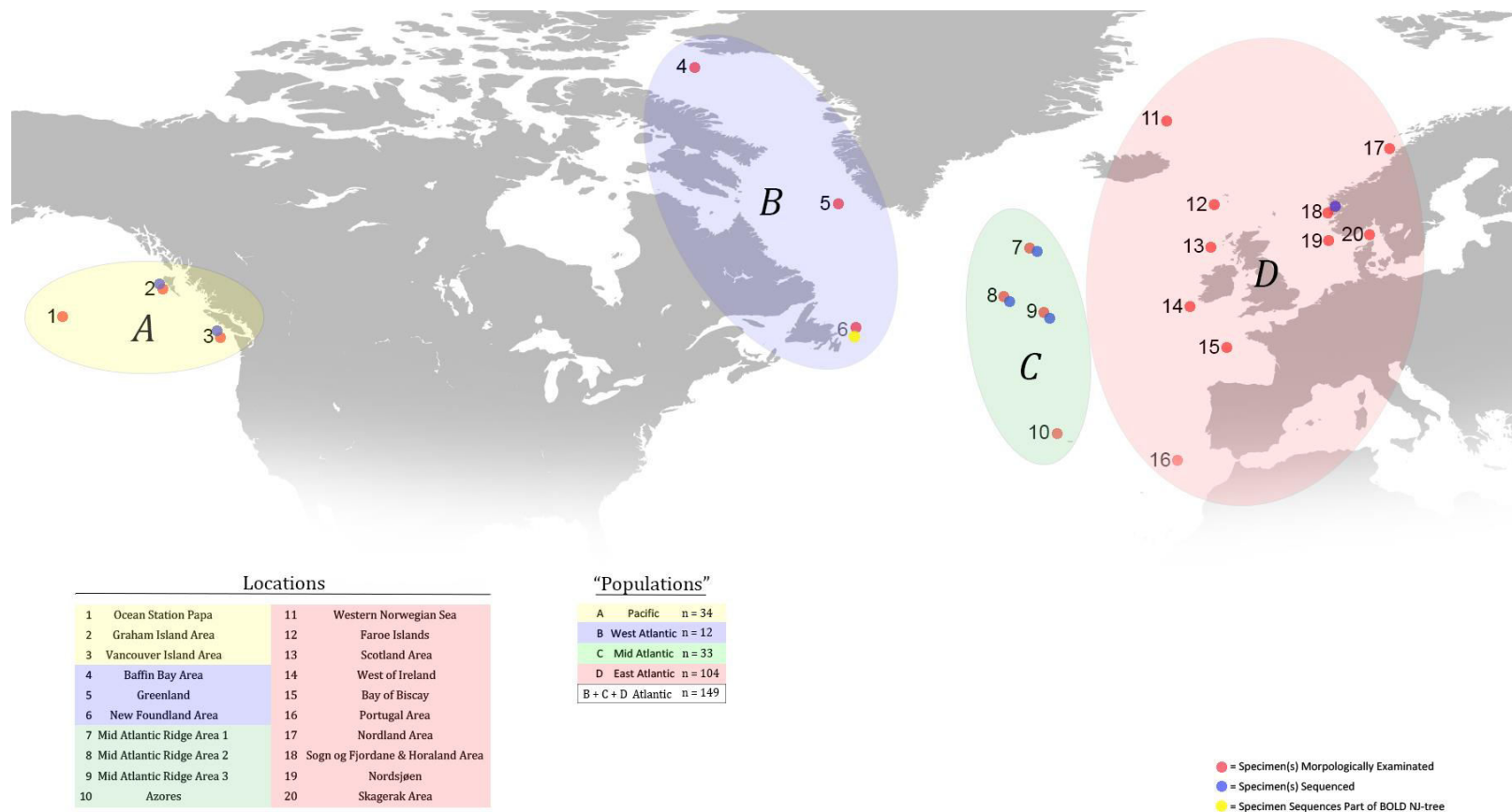
Figure 4.2.1. Map indicating the various geographical locations sampled, with geographic division labeled A) Pacific, B) West Atlantic, C) Mid Atlantic and D) East Atlantic. The divisions are color-coded yellow, blue, green and red, respectively. Red dots indicate specimen(s) investigated morphologically (with the sample sizes, n, for each group listed in the "Populations" box), blue dots indicate specimen(s) investigated genetically, and the yellow dot refers to the geographic origin of the sequences used as part of the NJ tree generated online (seen in figure A4.1. in Appendix 4). The map also shows that the geographic divisions are all sampled for both genetic and morphologic data.

4.2.2. *Pasiphaea tarda* – A polytypic species

The evidence presented in section 4.2.1 justified a merging of all morphological data from the Atlantic Ocean into one group. This enabled an analysis where the data from the Atlantic Ocean as a whole could be compared to the data from the Pacific Ocean. This increased the sample size of the Atlantic group, reducing the risk of sampling error influencing the results. The analyses conducted compared the Pacific group to the Atlantic group with regards to the following parameters: a) Spikes on ischium of the 2nd Pereiopod, b) Scaphocerite length and carapace length ratio, c) Total length and carapace length ratio, d) Width of scaphocerite (scaphocerite width/length) and e) Number of spikes on basis of the second pereiopod. No statistical significant (p>0.05) difference was found between the groups with regards to parameters a) or e). There was, nonetheless, an observed difference in the maximum value with regards to these parameters. The most common phenotype was 0 spikes on the basis of the second pereiopod for both populations, but the interval range was only 0-7 spikes in the Atlantic population, compared to 0-9 spikes in the Pacific population (see figure 3.7a). With regards to parameter a) the phenotype of having two spikes on the ischium was only documented in the Pacific population (see figure 3.4a and 3.7b). Both parameter a) and e) hint at the Pacific population having larger range values for parameters involving spikes on the segments of the 2nd pereiopod. Furthermore, referring to figure 3.7d, a higher number of spikes were also observed when comparing the number of spikes on the merus of the 2nd pereiopod, however, this finding is based on a very small sample size. The analysis conducted showed the scaphocerite length to carapace length ratio (parameter b) to be larger (p = 4,17x10^{-8}) for the Pacific population compared to the Atlantic population, i.e. the Pacific population has a shorter scaphocerite (lateral length) compared to the length of the carapace than the Atlantic population (see figure and table 3.4b). When comparing parameter c) Total length and carapace length ratio, it was found a statistical significant (p=0.0408) difference between the populations, with the Pacific population having a relatively larger carapace compared to the total length (see figure and table 3.4c). However, as discussed in section 4.3.1, this ratio is based on a

measurement prone to inaccuracy, wakening its credibility. Lastly, parameter d) Width of scaphocerite (scaphocerite width/length), was analyzed. The analysis revealed a significant difference (p = 2x10^{-16}), with the Pacific population having a relatively wider scaphocerite compared to the Atlantic population (see figure and table 3.4d). The analyses of the morphological data indicates that there are morphological difference the Pacific and Atlantic population of *P. tarda* with regards to the parameters investigated.

The phylogenetic tree in figure 3.1 placed 5 of the 6 sequences with origin in the Pacific in a distinct monophyletic group. This group had a bootstrap value of 99 and posterior probability of 1. The closest relative to this group was indicated to be the Atlantic group of *P. tarda,* with the node separating these groups having a bootstrap value of 75 and a posterior probability of 0.95. The node separating the divergent sequences (ID number: 010-00247-011), mentioned earlier, from the other Pacific sequences and the Atlantic clade had a bootstrap value of 94 and a posterior probability of 1. The distance analysis conducted (see table 3.1.) showed a mean K2P distances within the species of 1.36% (S.E. 0.25%). This is close to the high range of the spectrum (ranging from 0.285% to 1.375%) when compared to the mean K2P distances observed within species belonging to the Decapoda order (da Silva et al., 2011). This evidence indicate that although the phylogenetic tree in figure 3.1 show *P. tarda* consisting of 3 distinct clades, the divergence is not higher than observed for other species belonging to the same order. Limiting the analysis to data collected in the Atlantic Ocean, the analysis produces a mean K2P distance within the group of 0.04% (S.E. 0.02%). This is despite some of the Atlantic sequences being separated by 2000km, indicating that the Atlantic group of *P. tarda* is genetically homogenous. A distance analysis was also conducted comparing the Atlantic and Pacific groups to each other. The K2P distances produced corroborated the topology of the phylogenetic tree in figure 3.1, and it also indicated that the divergent sequence (ID number: 010-00247-011) was just as divergent from the other Pacific sequences, as it was divergent from the Atlantic sequences (see table 3.3). Another important circumstance is that the divergent sequence was extracted from a specimen collected at the same location (location 3 figure 4.2.1) as specimens belonging to

the larger monophyletic Pacific clade. This shows that the two Pacific lineages are coexisting, and not restricted to specific areas of the Pacific. The divergent specimen was also given an individual morphological examination, showing parameter values well within what is observed for the species as a whole (see raw data Appendix 1.4). The observed genetic divergence is therefore not perceivable in the specimen's morphology. In summary, the result from the genetic analyses indicates genetic divergence between the Atlantic and Pacific Ocean, with the data describing low genetic diversity within the Atlantic Ocean, and high genetic diversity within the Pacific Ocean.

Despite the analyses of the genetic data indicating divergent lineages within the *P. tarda* taxa, the degree of divergence is not indicative of *P. tarda* being a cryptic species, and all the lineages should be regarded as belonging to one species. The limited genetic variation within the Atlantic Ocean might be indicative of this population having its genetic diversity reduced through either a genetic bottleneck or the founder effect (Nei et al., 1975). The contrasting levels of genetic diversity between the specimens collected in the Pacific and the Atlantic Ocean is also indicative of limited or no gene flow between the oceans. This provides evidence demonstrating that the Atlantic and Pacific populations are both geographically and genetically separated. Regarding the populations as geographically distinct sub-specie of *P. tarda* might therefore be appropriate. This proposal is substantiated by the results from the statistical analysis of the morphological data, documenting observable differences between the populations on a population level, as well as giving indications of some phenotypes only being present in the Pacific. The failure to observe certain phenotypes in the Atlantic Ocean can possibly accredited to the loss of the genes producing these phenotypes through a genetic bottleneck or the founder effect. Pragmatic arguments could also be made supporting the establishment of *P. tarda* as a polytypic species. The two sub-species could more easily be described separately, making the respective descriptions less general. Having less general descriptions would make the populations more distinguishable from other species. E.g. according to the Global Biodiversity Information Facility (GBIF) *P. tarda* is coexisting with the closely related *P. multidentata* in the Atlantic Ocean

(Gbif.org, 2016). Current identification keys distinguish the two species by a comparison of the number of spikes on the basis segment of the 2nd pereiopod. *P. multidentata* has between 7-12 spikes on the basis of the 2nd pereiopod (Christiansen, 1972), compared to 0-7 for *P. tarda* in the Atlantic Ocean. However, the data examined for this thesis documents *P. tarda* having both 8 and 9 spikes on the basis of the 2nd pereiopod, increasing the overlap between the two species. However, this phenotype is, according to available data, limited to the Pacific Ocean. Making *P. tarda* a polytypic species would mean that these characters would not apply to the Atlantic sub-species, and the described morphologic overlap between *P. tarda* and *P. multidentata* in the Atlantic Ocean would remain small, making it easier to distinguish the two species. The two geographically separated sub-species of *P. tarda* should be classified under the trinomens *Pasiphaea tarda atlanticus* and *Pasiphaea tarda pacificus*, with the last parts of the name referring to the respective oceans they inhabit.

4.2.3 The synonymization of *Pasiphaea princeps* with *P. tarda*

In 1882 Sidney I. Smith described the new species *Pasiphaea princeps*. The description is based on a single large (total length 235mm) female specimen (ID 5473, Appendix 1.4) collected off the coast of the northeastern United States close to location 21 in figure 2.1 (Smith, 1882). At several parts of the description Smith remarks upon the resemblance between the new species and *P. tarda,* but proceeds by describing distinguishing characters. Smith (1882) claims that the antenna, antennule and all oral appendages are similar in both species, yet the tip of the scaphocerite ends in a lamellar tooth in *P. princeps*, distinguishing the new species from *P. tarda*. However, by visually inspecting the material collected for this thesis, this assertion is refuted. Smith (1882) continues by describing the pereiopods, again remarking the similarity between *P. princeps* and *P. tarda.* Though, the text does not provide an account of what the distinguishing characters are, a comparison of the original descriptions by Smith (1882) and Krøyer (1845), some distinctions becomes apparent. The number of spikes on the basis of the 2nd pereiopod is 0 for *P. princeps,* but 3 for *P. tarda,* but this distinction is countered by results presented in this thesis, where data indicate that 0 is actually the most common phenotype of this character for *P. tarda* (see

figure 3.7a). Furthermore, the number of spikes on the merus of the 1st pereiopod is described to be 0 for *P. princeps* and 5 for *P. tarda*. This distinction is also refuted by data collected for this thesis, with 0 spikes on the merus of the 1st pereiopod being seen in a specimen of *P. tarda* (ID 211015 2 split from ZMBNU 84087, see Appendix 1.4) collected at the Mid Atlantic Ridge. This specimen was additionally identified as *P. tarda* by genetic data (see phylogenetic tree in figure 3.1), verifying the specimen's taxonomic belonging. The number of spikes present on the merus of the 2nd pereiopod is also differing in the two descriptions. *P. princeps* is described of having 5 spikes, while *P. tarda* is described as having 12 spikes. None of the *P. tarda* specimens examined for this character had a matching (or lower) number of spikes. However, only specimens with genetic data confirming the specimens as *P. tarda* were examined for the number of spikes on the merus segments, limiting the sample size to only 17 specimens. Nonetheless, the specimen earlier mentioned having 0 spikes on the merus of the 1st pereiopod, had 6 spikes on the merus of the 2nd pereiopod, very close to 5 spikes as seen in the type of *P. princeps.* Figure 3.7. c) and d) illustrates that this character is variable, and with a larger sample size it is plausible that this phenotype would be documented in *P. tarda* as well. Smith (1882) describes the rostrum as being "obliquely upturned", an observation confirmed by visual inspection of the type specimen. This is not a distinguishable character between the two species *per se*, but as seen in figure 3.6, having an upturned rostrum is very unusual for a *P. tarda* specimen of comparable size (total length 235mm). However, in this study all rostrums were assigned to one of four categories: "Spiky Curved Up", "Curved Up", "Straight" and "Curved Down" (specimens infected with Ellobiopsidae was assigned to group "NA"). Designing the analysis in this way does not reflect the high variation of shapes seen in this character. Pasiphaeids are also frequently infected by the parasitic dinoflagellates belonging to the Ellobiopsidae family, resulting in a distorted shape of the rostrum (Hoffman and Yancey, 1966, Sivertsen and Holthuis, 1956). Approximately 7% of the specimens of P. tarda examined for this thesis were visibly infected. The variability of this character, further increased by distorting parasitic infections, makes this character highly unreliable and unsuitable to be used as a distinguishing character. Sivertsen and Holthuis (1956) discuss the

taxonomic status of *P. tarda, P. principalis,* and *P. princeps*. The paper concludes by suggesting a synonymization of all three species, which in the case of *P. tarda* and *P. principalis* a synonymization has been implemented. The conclusion of synonymization of *P. princeps* with *P. tarda* is based on failing to find morphological differences of taxonomic importance. The authors refute the validity of distinguishing characters presented by (Sund, 1913). Sund (1913) claims that the two species can be distinguished be the following characters a) Egg size, b) Number of spikes on the 2nd pereiopod, and c) The comparative length of the 4th and 5th pereiopod. By comparing material of *P. tarda* with the type specimen of *P. princeps* all three arguments are refuted. Argument b) is also disproven by this thesis. Iwasaki (1990) comments on the paper by Sivertsen and Holthuis (1956), disagreeing in the synonymization of *P. tarda* and *P. princeps*. The author lists 6 characters distinguishing the two species, functioning as arguments for keeping the species separate: a) The carapace is not carinate in *P. princeps,* while dorsally carinate in *P. tarda,* b) The merus of the first pereopod is without a spine in *P. princeps,* while it has 2-8 spines in *P. tarda,* c) The merus of the second pereopod has five spines in *P. princeps,* while 14-21 spines in *P. tarda,* d) The ischium of the second pereopod is without a spine in *P. princeps,* while it has 0 or 1 spine in *P. tarda,* e) The basis of the second pereiopod is without a spine in *P. princeps,* while 2-7 spines are present in *P.* tarda, and f) The carapace length of the ovigerous female is 75mm in *P. princeps*, while 39 and 41.5 mm in *P. tarda.* Argument b), c) and e) were invalidated earlier in this section. Argument d) invalidates itself by being contradictory, and is further refuted by data collected for this thesis (see figure 3.7b). Character a) is, as many of the other characters found in *P. tarda*, highly variable. Nevertheless, a carapace with no dorsal carina is observed in *P. tarda* (e.g. specimen 051215-8), consequently refuting argument a). Argument f) "The carapace length of the ovigerous female is 75 mm in *P. princeps*, while 39 and 41.5 mm in *P. tarda*" is highly flawed. The argument is based on the assumption that one data point is representative of the species *P. princeps*, and two data points being representative of the species *P. tarda*. The size of ovigerous is observed to be highly variable in the related species *P. sivado* and *P. multidentata* (Company et al., 2001), and it is therefore reasonable to assume that variation within this

character would be observed for *P. princeps* and *P. tarda* as well. This argument can therefore be discounted. Similarly to Sivertsen and Holthuis (1956) no characters of taxonomic importance have been identified, and those characters suggested by previous others as been invalidated. If the two species remain taxonomically distinct they would form a morphological indistinguishable sympatric species complex, both living in the Atlantic Ocean, present in the same depth strata. This is unlikely due to the "Competitive Exclusion Principle", which usually leads to niche shifts and the facilitation of divergent evolution (Hardin, 1960). Sympatric cryptic species have, nonetheless, been documented in the past (Rugman-Jones et al., 2010, Baker, 1984) lending credence to the theory. Due to the similar morphological appearance of cryptic species, molecular approaches are often the most effective way to identify and distinguish species belonging to the same complex. This is not a feasible means of distinguishing *P. tarda* and *P. princeps* due to the old age and degraded DNA of the *P. princeps* type (ID: 5473). The taxonomical distinction of these two species will therefore have to be based on morphological differences, which have yet to be identified. On the contrary, all proposed distinguishing characteristics have been refuted, rendering the *status quo* unlikely and unparsimonious. A synonymization of *Pasiphaea princeps* with *Pasiphaea tarda* is therefore recommended.

4.3. Minor results

4.3.1. Age influenced morphological changes in *P. tarda*

Statistical analyses were conducted to reveal age influenced morphological changes in selected characters, with the presumption of age being reflected in size. The carapace length was chosen as the size parameter rather than the total length. This was due to the inaccuracy of the latter measurement discussed in section 4.1.3. Correlation tests revealed a positive correlation between age (carapace length) and parameter 1) Scaphocerite width, 2) Number of spikes on basis of the 2nd pereiopod, and 3) Number of spikes on the ischium of the 2nd pereiopod. The correlations were, 0,237, 0,340 and 0,131, respectively, i.e. the scaphocerite gets wider, and the amount of spikes on the basis and ischium segments of the 2nd pereiopod increases with age (see figure 3.5a-c). Regardless,

the correlations detected are only applicable when describing the species as a whole, and cannot aptly be used to predict the future phenotype of a specimen at later life stages. E.g. if a specimen has 0 spikes on the basis of the 2nd pereiopod as a juvenile, it still may lack spikes on this segment when it is fully grown. Furthermore, the categorical data documenting the types of rostrums for the specimens examined were also analyzed. The analysis revealed that this character as also showed signs of morphological change related to size (see figure 3.6). The general trend showed that the rostrum evolves from being more upward pointing in earlier life stages, to gradually straightening out, and in some cases becoming bent down at late life stages. Nevertheless, the data also shows great variety of this character trait at all life stages examined, except in very large of very small individuals (see figure 3.6). Age influenced morphological changes are common for metazoans, and is also documented in crustaceans (Petrov and Marincek, 1995). A morphological description of any organism, including Pasiphaeids, should therefore consider both the variability in character traits, as well as the change in morphology when the organism grows/ages. Any statistical analysis comparing morphology between groups should also take these factors into account, making sure that any observed difference is attributed to real differences, and that they are not due to differences in size the distribution of specimen within data sets. The statistical models used for the analyses of the morphological data therefore included size as a covariate. The variability of morphology within species, as well as the lack of morphological differences between certain species, e.g. cryptic species, demonstrate some of the advantages barcodes have over traditional morphological analysis (Hills, 1987). This advantage is accredited to that the analysis of barcodes are not affected by age influenced morphological changes, plasticity or variability. The advantages of barcodes are exemplified in the study by Pramual and Wongpakam (2014), linking unknown larval life stages of black flies (family: Simuliidae) to known species described in their adult form. That fact that statistical analyses performed as part of this thesis documents age influenced morphological changes is therefore not surprising. Nonetheless, the uncovered knowledge about the degree of correlation, if the correlation is negative or positive, and

knowledge about which characters are influenced by age, are valuable knowledge describing defining characteristics of *P. tarda*.

4.3.2. Sequences possibly belonging to an undescribed species

The preliminary phylogenetic tree seen in figure 1.1 indicated that two genetically distinct clades of *P. tarda* inhabit the Atlantic Ocean. One of the groups consisted of sequences derived from specimen of *P. tarda* collected in the Sognefjord, while the other group consisted of three sequences downloaded from GenBank.com. According to the GenBank voucher these sequences were collected at Rosemary Bank northwest of Scotland, and determined to be *P. tarda* (Ncbi.nlm.nih.gov, 2016). The topology of the preliminary phylogenetic tree (figure 1.1) was confirmed in the phylogenetic tree seen in figure 3.1. However, the branch length indicated a high degree of divergence between the Rosemary Bank lineage and both the Pacific and Atlantic populations of *P. tarda,* undermining the existing species identification. A distance analysis of the sequences collected at Rosemary Bank (referred to as *P. sp.* in figure 3.1) produced a mean K2P distance between this group and its closest genetic match, the Atlantic population of *P. tarda,* of 7.27% (S.E. 1.19%). This is approximately the same K2P distance found between *P. multidentata* and *P. tarda*, further reducing the credibility of the existing species identification. A comparison of the K2P distance between *P. tarda*, and the now plausibly unidentified species, *P. sp.*, with K2P distances normally found within the order of Decapoda (da Silva et al., 2011) corresponds to a K2P distance normally found between species within the same genus, corroborating the previous conjecture. It is unknown if the sequences belong to an undescribed taxon, or if the lack of matching sequences in GenBank is due to lack of data. One of the specimens from whom a sequence was extracted was obtained, but the specimen was highly degraded and ill suited for a positive species determination. Although, no positive species determination was achieved, this finding reveals some of the weaknesses associated with GenBank, and serves as an example of why scientists should be skeptical of

results based on morphological or molecular data presented without corroborating evidence.

4.3.3. Time since divergence between the Atlantic and Pacific Populations

The cladogram in figure 3.2 has time estimates indicating the time since divergence (in myr) located at each node. The estimated time of divergence between the *P. tarda* clade and the unidentified species, *P. sp.*, collected at Rosemary Bank northwest of Scotland, is indicated to have taken place about 2.93 mya. The estimated time since divergence between the Pacific and Atlantic population is estimated to have taken place about 0.98 mya, while the split up of the mitochondrial lineage separating the divergent sequence (ID number: 010-00247-011) from the Pacific from the two other *P. tarda* groups is estimated to have taken place about 1.34 mya. These time estimates are based on a strict clock approach with the mutation rate of 0.014/Myr (Knowlton and Weight, 1998). No empirical mutation rate is estimated for the COI gene for Pasiphaeids, so a mutation rate was derived from a study by Knowlton and Weight (1998). The authors used the specified mutation rate to estimate the time of divergence between 15 sister-species of snapping shrimps separated during the formation of the Isthmus of Panama. Snapping shrimp belong to the same infraorder (Caridea) as Pasiphaeids, and the estimated time since divergence is based on the assumption that this mutation rate is comparatively similar to the mutation rate for the COI gene in Pasiphaeids. According to Gbif.org (Gbif.org, 2016) *P. tarda* is mainly present in the oceans of the northern hemisphere, leaving few possible routes of dispersal between the Pacific and Atlantic Oceans; Via the Isthmus of Panama prior to its final formation, or via trans-Arctic dispersal. The estimated time of divergence (2.93mya) between the *P. tarda* clade and the unidentified species coincides with the separation of the Pacific Ocean and the Caribbean by the formation of the Isthmus of Panama, providing a possible mechanism facilitating the division of these two groups. However, it is unlikely that this marine regression event provides a satisfactory explanation to the mechanism that separated the Pacific and Atlantic population of *P. tarda* due to temporal incompatibility. The two populations got separated about 1 mya, which is approximately 2 myr after the formation of the Isthmus of Panama. As mention

in section 4.2.2, one plausible explanation to the limited genetic variation observed in the Atlantic population could be attributed to the bottleneck effect. This explanation is based on a presumption where the Pacific Population colonized the Atlantic Ocean via a trans-Arctic dispersal event about 1 mya. Jakobsson et al. (2016) estimates that the ice sheet covering the Atlantic Ocean during MIS 6 (about 1.91 kya) had an average thickness of 1121 meters below present day sea level. The authors suggests that glaciation events like this, covering the entire Central Arctic Ocean Basin, has likely taken place several times during the Quaternary Glaciation period (2.58 mya – present), a period coinciding with the assumed colonization event. Such glaciation events, with deeply penetrating sea ice, would be a natural abiotic barrier limiting dispersal of north Pacific and Atlantic species. Nevertheless, periods where the ice sheet was thin or absent would allow for colonization via the route described. Through parsimonious reasoning this theory would also imply that *P. tarda* is of Pacific origin. The mechanisms apparently keeping the populations separate today are unknown, but the Arctic route suggested as the route of dispersal is a known barrier separating Pacific and Atlantic organisms (Wisz et al., 2015). Nonetheless, the latter hypothesis provides a plausible mechanism explaining both the separation of the unidentified species, *P. sp.*, as well as the separation of the Atlantic and Pacific populations of *P. tarda*. This same mechanism of separation can also be used to explain the separation of the clade compromised of *P. tarda* and *P. sp. a*nd the clade compromised of *P. multidentata* (see figure 3.1), providing an example of vicariant speciation. However, any phylogenetic divergence prior to the opening of the Bering Strait is not compatible with this theory.

5.0. Conclusion and final remarks

The present study has provided a phylogenetic reconstruction of the *Pasiphaea* genus including 8 of the 70 excepted species. Furthermore, analyses of morphological data, substantiated by genetic analyses, have resulted in a proposed division of the *Pasiphaea tarda* taxon into two sub-species: *Pasiphaea tarda atlanticus* in the Atlantic Ocean, and *Pasiphaea tarda pacificus* in the Pacific Ocean. The estimated time since divergence between the two sub-species was estimated to have taken place about 1 mya, and can possibly be attributed to a colonization event of the Atlantic Ocean via trans-Arctic dispersal from the Pacific Ocean. Morphological examination revealed no differences of taxonomic importance between the sympatric species *P. princeps* and *P. tarda*, resulting in a proposed synonymization. Statistical analysis of the morphological data also documented age influenced morphological change in the *P. tarda* taxon, and provides new range values for several morphological parameters.

Sequences determined to be *P. tarda,* collected at Rosemary Bank northwest of Scotland, were downloaded from GenBank.com. However, the K2P distances produced indicated divergences in accordance with these sequences belonging to a distinct species. According to gbif.org, there are at least 7 species inhabiting the North Atlantic Ocean not accounted for in the phylogenetic tree created as part of this thesis. The sequences collected at Rosemary Bank may possibly belong to any of these 7 taxa, or plausibly any other member of the *Pasiphaea* genus not documented by Gbif.org. The present study also revealed that many distinguishing characters of *P. tarda* were much more variable than previously believed. This is likely the case for many other species, and these findings demonstrate the value of having comparable sequences (barcodes) stored in a database (such as GenBank) tied to one or more voucher specimens. Such a database is an invaluable resource in the taxonomic determination of unknown specimens like the three specimens collected at Rosemary Bank, as well as revealing possible species complexes and/ cryptic species.

Acknowledgments

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Appendix 1 – Specimen List

1.1 Specimens sequenced

Table A1.1. Overview of all specimens that have been sequences as part of this thesis providing information about the species, it's unique ID-number, where the specimen was collected, it's fixative medium, and what primer(s) was used when sequencing the specimen.

#	Species	ID	Split From	Collect Institution	Collect Country	Collect Area	Latitude / Longitude (DMS)	Station number	Collection Date	Fixative	Primer
1	*Pasiphaea multidentata*	ZMBN - U 3972	-	Universitetsmuseet i Bergen	Norway	Sognefjorden	61°9.518836' N 7°16.339402' E		17.11.2012	96% EtOH	LCO/HCO
2	*P. multidentata*	ZMBN - U 4024	-	Universitetsmuseet i Bergen	Norway	Sognefjorden	61°08.278087' N 5°48.937860' E		16.11.2012	96% EtOH	LCO/HCO
3	*P. sivado*	ZMBN - U 4049	-	Universitetsmuseet i Bergen	Norway	Sognefjorden	61°08.598098' N 6°53.765028' E		17.11.2012	96% EtOH	LCO/HCO
4	*P. sivado*	ZMBN - U 4799	ZMBN - U 4245	Universitetsmuseet i Bergen	Norway	Sognefjorden - Sognesjøen			01.11.2011	96% EtOH	LCO/HCO
5	*P. tarda*	ZMBN - U 3971	-	Universitetsmuseet i Bergen	Norway	Sognefjorden - Aurlandsfjorden	60°54.512127' N 7°09.841466' E		17.11.2012	96% EtOH	LCO/HCO
6	*P. tarda*	ZMBN - U 3988	-	Universitetsmuseet i Bergen	Norway	Sognefjorden	61°7.2205' N 6°54.5222' E		3.1011	96% EtOH	LCO/HCO
7	*P. tarda*	ZMBN - U 4463	-	Universitetsmuseet i Bergen	Norway	Sognefjorden	61°5.906028' N 65°8.65555'6 E		27.05.2013	96% EtOH	LCO/HCO
8	*P. tarda*	ZMBN - U 4800	ZMBN - U 3989	Universitetsmuseet i Bergen	Norway	Lustrafjorden - Nattropefjorden	61°24.721684' N 7°27.74791'3 E		8.11.2012	96% EtOH	LCO/HCO
9	*P. tarda*	ZMBN - U 4326	-	Universitetsmuseet i Bergen	Norway	Sognefjorden - Sognesjøen	61°0.8282' N 4°53.3246' E	HM2011-11-40AG	4.11.2011	96% EtOH	LCO
10	*P. tarda*	ZMBN - U 3990	-	Universitetsmuseet i Bergen	Norway	Sognefjorden - Lustrafjorden	61°14.8226' N 7°21.8388' E	HM2011-11-31AG	3.11.2011	96% EtOH	LCO
11	*P. multidentata*	ZMBN - U 4183	-	Universitetsmuseet i Bergen	Norway	Sognefjorden - Nordresvik	61°8.294905' N 5°45.643471' E	HM2013-05-16AG	28.05.2013	96% EtOH	LCO
12	*P. mutidetata*	100915-2	ZMBN - U 4141	Universitetsmuseet i Bergen	Norway	Sognefjorden - Lustrafjorden	61°21.885383' N 7°22.799310' E	HM2013-05-01RT	26.05.2013	96% EtOH	LCO
13	*P. mutidetata*	100915-3	ZMBN - U 4141	Universitetsmuseet i Bergen	Norway	Sognefjorden - Lustrafjorden	61°21.885383' N 7°22.799310' E	HM2013-05-01RT	26.05.2014	96% EtOH	LCO
14	*P. mutidetata*	100915-4	ZMBN - U 4141	Universitetsmuseet i Bergen	Norway	Sognefjorden - Lustrafjorden	61°21.885383' N 7°22.799310' E	HM2013-05-01RT	26.05.2015	96% EtOH	LCO
15	*P. mutidetata*	100915-5	ZMBN - U 4145	Universitetsmuseet i Bergen	Norway	Sognefjorden - Lustrafjorden	61°21.108093' N 7°22.207150' E	HM2013-05-02RT	26.05.2013	96% EtOH	LCO

#	Species	ID	Split From	Collect Institution	Collect Country	Collect Area	Latitude / Longitude (DMS)	Station number	Collection Date	Fixative	Primer
16	*P. mutidetata*	100915-6	ZMBN - U 4145	Universitetsmuseet i Bergen	Norway	Sognefjorden - Lustrafjorden	61°21.108093' N 7°22.207150' E	HM2013-05-02RT	26.05.2014	96% EtOH	LCO
17	*P. mutidetata*	100915-7	ZMBN - U 4145	Universitetsmuseet i Bergen	Norway	Sognefjorden - Lustrafjorden	61°21.108093' N 7°22.207150' E	HM2013-05-02RT	26.05.2015	96% EtOH	LCO
18	*P. mutidetata*	*Førde Indre 1*	-	Universitetsmuseet i Bergen	Norway	Førdefjorden - Indre				96% EtOH	LCO
19	*P. mutidetata*	*Førde Ytre 1*	-	Universitetsmuseet i Bergen	Norway	Førdefjorden - Ytre				96% EtOH	LCO
20	*P. mutidetata*	*Førde Ytre 2*	-	Universitetsmuseet i Bergen	Norway	Førdefjorden - Ytre				96% EtOH	LCO
21	*P. mutidetata*	*Førde Ytre 3*	-	Universitetsmuseet i Bergen	Norway	Førdefjorden - Ytre				96% EtOH	LCO
22	*P. mutidetata*	*Førde Ytre 4*	-	Universitetsmuseet i Bergen	Norway	Førdefjorden - Ytre				96% EtOH	LCO
23	*P. mutidetata*	*Førde Ytre 5*	-	Universitetsmuseet i Bergen	Norway	Førdefjorden - Ytre				96% EtOH	LCO
24	*P. mutidetata*	*Førde Ytre 6*	-	Universitetsmuseet i Bergen	Norway	Førdefjorden - Ytre				96% EtOH	LCO
25	*P. tarda*	*021015-2*	ZMBN - U 4145	Universitetsmuseet i Bergen	Norway	Sognefjorden - Lustrafjorden	61°21.108093' N 7°22.207150' E	HM2013-05-02RT	26.05.2013	96% EtOH	LCO
26	*P. tarda*	*021015-3*	ZMBN - U 4145	Universitetsmuseet i Bergen	Norway	Sognefjorden - Lustrafjorden	61°21.108093' N 7°22.207150' E	HM2013-05-02RT	26.05.2013	96% EtOH	LCO
27	*P. tarda*	051015-1	ZMBN - U 4141	Universitetsmuseet i Bergen	Norway	Sognefjorden - Lustrafjorden	61°21.885383' N 7°22.799310' E	HM2013-05-01RT	26.05.2013	96% EtOH	LCO
28	*P. tarda*	051015-2	ZMBN - U 4137	Universitetsmuseet i Bergen	Norway	Sognefjorden - Lustrafjorden	61°21.108093' N 7°22.207150' E	HM2013-05-02RT	26.05.2013	96% EtOH	LCO
29	*P. multidentata*	141015-3	-	Universitetsmuseet i Bergen	Norway	Masfjorden	60°52.485973' N 5°25.746584' E	-	09.22.2015	96% EtOH	LCO
30	*P. tarda*	201015-2	LS 12300	Universitetsmuseet i Bergen	-	Mid-Atlantic Ridge	48°0'10.8'' N 29°34'13.8'' W	-	25.06.2004	96% EtOH	LCO
31	*P. tarda*	201015-3	LS 12300	Universitetsmuseet i Bergen	-	Mid-Atlantic Ridge	48°0'10.8'' N 29°34'13.8'' W	-	25.06.2004	96% EtOH	LCO
34	*P. tarda*	ZMBN - U 3989	-	Universitetsmuseet i Bergen	Norway	Lustrafjorden - Nattropefjorden	61°24.721684' N 7°27.747913' E	HM2012-11-21RT	8.11.2012	96% EtOH	LCO
36	*P. tarda*	010-00247-011		Royal British Columbia Museum	Canada	British Columbia Pacific Ocean	48°22.099' N 126°27.748' W	-	29.08.2001	70% EtOH	LCO
37	*P. sivado*	121115-1	ZMBN - U 4457	Universitetsmuseet i Bergen	Norway	Sognefjorden	61°12.243948' N 7°5.899001' E	RT09	27.05.2013	96% EtOH	LCO

#	Species	ID	Split From	Collect Institution	Collect Country	Collect Area	Latitude / Longitude (DMS)	Station number	Collection Date	Fixative	Primer
38	P. sivado	121115-2	ZMBN - U 4457	Universitetsmuseet i Bergen	Norway	Sognefjorden	61°12.243948' N 7°5.899001' E	RT09	27.05.2013	96% EtOH	LCO
39	P. tarda	131115-1	ZMBN U-4154	Universitetsmuseet i Bergen	Norway	Sognefjorden	61°7.469380' N 5°41.351488' E	HM2013-05-IK17	28.05.2014	96% EtOH	LCO
40	P. sivado	201115-1	ZMBN U-4488	Universitetsmuseet i Bergen	Norway	Fjærlandsfjorden	61°19.398720' N 6°41.573402' E	HM2012-11-14AG	27.05.2013	96% EtOH	LCO
41	P. sivado	231115-1	ZMBN U-4488	Universitetsmuseet i Bergen	Norway	Fjærlandsfjorden	61°19.398720' N 6°41.573402' E	HM2012-11-14AG	27.05.2014	96% EtOH	LCO
42	P. tarda	291015-1	010-00260-004	Royal British Columbia Museum	Canada	British Columbia Pacific Ocean	48°19.947' N 126°23.746' W	-	03.09.2001	70% EtOH	LCO
43	P. tarda	211015-2	ZMBN - U 84087	Universitetsmuseet i Bergen	-	Mid-Atlantic Ridge	52°58' N 34°52' W	MAR-ECO stn 70-385-1167	26.07.2004	96% EtOH	LCO
44	P. tarda	201015-1	LS 8900	Universitetsmuseet i Bergen	-	Mid-Atlantic Ridge	57°3'3.96'' N 31°12'58.32'' W	-	13.06.2004	96% EtOH	-
38	P. sivado	121115-2	ZMBN - U 4457	Universitetsmuseet i Bergen	Norway	Sognefjorden	61°12.243948' N 7°5.899001' E	RT09	27.05.2013	96% EtOH	LCO
39	P. tarda	131115-1	ZMBN U-4154	Universitetsmuseet i Bergen	Norway	Sognefjorden	61°7.469380' N 5°41.351488' E	HM2013-05-IK17	28.05.2014	96% EtOH	LCO
40	P. sivado	201115-1	ZMBN U-4488	Universitetsmuseet i Bergen	Norway	Fjærlandsfjorden	61°19.398720' N 6°41.573402' E	HM2012-11-14AG	27.05.2013	96% EtOH	LCO
41	P. sivado	231115-1	ZMBN U-4488	Universitetsmuseet i Bergen	Norway	Fjærlandsfjorden	61°19.398720' N 6°41.573402' E	HM2012-11-14AG	27.05.2014	96% EtOH	LCO

1.2 Sequences download from GenBank

Table A1.2. Overview of all sequences downloaded from GenBank, providing information about the species, the GenBank accession number, and the geographical area the sequence is derived from.

Species	Source	Accession number	Collect Country	Latitude / Longitude (DMS)	Collect Area
P. sivado	GenBank	KP759486	France	44°37'59.88'' N 1°55'0.012'' W	West Coast France
P. sivado	GenBank	KP759487	France	44°37'59.88'' N 1°55'0.012'' W	West Coast France
P. sivado	GenBank	JQ306263	Portugal	36°47'60.0'' N 7° 46'12.0'' W	South Coast Portugal
P. sivado	GenBank	JQ306262	Portugal	36°47'60.0'' N 7° 46'12.0'' W	South Coast Portugal
P. sivado	GenBank	JQ306265	Portugal	36°47'60.0'' N 7° 46'12.0'' W	South Coast Portugal
P. sivado	GenBank	KP759486	France	44°37'59.88'' N 1°55'0.012'' W	West Coast France
P. planidorsalis	GenBank	KP759482	New Caledonia	20°54'35.28'' S 165°35'60.0'' E	East Coast
P. planidorsalis	GenBank	KP759481	New Caledonia	19°48'55.6308'' S 158°58'20.1144'' E	West Coast - Coral Sea
P. telacantha	GenBank	KP759492	New Caledonia	23°19'9.48'' S 167°58'37.2'' E	South Coast
P. telacantha	GenBank	KP759491	New Caledonia	23°2'48.12'' S 166°52'30.0'' E	South Coast
P. telacantha	GenBank	KP759490	New Caledonia	21°4'59.88'' S 165°50'2.4'' E	East Coast
P. hoplocerca	GenBank	JQ306169	Portugal	36°32'60.0'' N 9°4'12.0'' W	South Coast
P. pacifica	GenBank	DQ882133	Canada	-	West Coast - British Columbia
P. pacifica	GenBank	DQ882131	Canada	-	West Coast - British Columbia
P. pacifica	GenBank	DQ882135	Canada	-	West Coast - British Columbia
P. pacifica	GenBank	DQ882134	Canada	-	West Coast - British Columbia
P. pacifica	GenBank	DQ882132	Canada	51°32'24.0'' N 128°12'36.0'' W	West Coast - British Columbia
P. multidentata	GenBank	FJ581853	Canada	49°20'26.88'' N 64°29'25.08'' W	East Coast - Quebec
P. multidentata	GenBank	JQ305977	Scotland	59°15'36'' N 10°0' W	North West - Rosemary
P. tarda	GenBank	JQ305981	Scotland	59°12'36.0'' N 10°0' W	North West - Rosemary
P. tarda	GenBank	JQ305980	Scotland	59°12'36.0'' N 10°0' W	North West - Rosemary
P. tarda	GenBank	JQ305979	Scotland	59°12'36.0'' N 10°0' W	North West - Rosemary
P. tarda	GenBank	DQ882137	Canada	53°25'12.0'' N 133°14'24'' W	West Coast - British Columbia

Species	Source	Accession number	Collect Country	Latitude / Longitude (DMS)	Collect Area
P. tarda	GenBank	DQ882139	Canada	-	West Coast - British Columbia
P. tarda	GenBank	DQ882136	Canada	53°25'12.0'' N 133°14'24'' W	West Coast - British Columbia
P. tarda	GenBank	DQ882138	Canada	-	West Coast - British Columbia

1.3 Specimens Examined Morphologically – Raw Data

Table A1.3. Overview of all specimens of *P. tarda* examined morphologically along with a unique ID-number, it's geographical belonging and it's corresponding parameter values for the characters examined in this paper.

Species	ID	Split From	Collect institute	Location	Country	Station	Latitude/Longitude (DMS)	Date	Length cm (telson to rostrum)	Length Carapace mm (dorsal side to eye socket)	Spikes on Basis Left 2nd Pereiopod	Spikes on Basis Right 2nd Pereiopod	Spikes on Left Ischium 2P	Spikes on Right Ischium 2P	Lateral Length Scaphocerite	Width Scaphocerite	Rostrum
Pasiphaea tarda	100915-7	ZMBN U 4145	Universitetsmuseet i Bergen	Sognefjorden - Lustrafjorden	Norway	-	-	-	12	36,3	1	2	0	NA	14,8	3,8	Straight
P. tarda	ZMBN U-4800	-	Universitetsmuseet i Bergen	Sognefjorden - Lustrafjorden	Norway	-	-	-	12,5	36	2	2	0	0	15,5	4,4	Curved Up
P. tarda	ZMBN U-3990	-	Universitetsmuseet i Bergen	Sognefjorden - Lustrafjorden	Norway	-	-	-	7,5	19,8	1	1	0	0	8,7	2,5	Curved Up
P. tarda	021015-1	ZMBN U 4145	Universitetsmuseet i Bergen	Sognefjorden - Lustrafjorden	Norway	-	-	-	8,5	30	2	3	NA	NA	13	3,3	Straight
P. tarda	021015-2	ZMBN U 4145	Universitetsmuseet i Bergen	Sognefjorden - Lustrafjorden	Norway	-	-	-	9,5	29,8	1	1	0	0	12,2	3,4	Curved Up
P. tarda	021015-3	ZMBN U 4145	Universitetsmuseet i Bergen	Sognefjorden - Lustrafjorden	Norway	-	-	-	13	40	0	0	0	0	17,2	4,3	Straight
P. tarda	051015-2	ZMBN U 4137	Universitetsmuseet i Bergen	Sognefjorden - Lustrafjorden	Norway	-	-	-	9,5	29,9	2	1	0	0	13	3,3	Curved Up
P. tarda	051015-1	ZMBN U 4141	Universitetsmuseet i Bergen	Sognefjorden	Norway	-	-	-	11	38,5	1	1	0	0	NA	NA	Straight
P. tarda	ZMBN U-4463	-	Universitetsmuseet i Bergen	Sognefjorden	Norway	-	-	-	6,5	21,4	0	0	0	0	9,3	2,5	Straight
P. tarda	ZMBN U-3989	-	Universitetsmuseet i Bergen	Sognefjorden	Norway	-	-	-	13	37,2	1	2	0	0	15,8	4,4	Straight
P. tarda	ZMBN U-3971	-	Universitetsmuseet i Bergen	Sognefjorden	Norway	-	-	-	7	21,2	2	4	NA	NA	9,4	2,3	Straight
P. tarda	ZMBN U-3988	-	Universitetsmuseet i Bergen	Sognefjorden	Norway	-	-	-	8,5	25,4	2	0	0	0	10,6	2,9	Straight
P. tarda	ZMBN U-4456	-	Universitetsmuseet i Bergen	Sognefjorden	Norway	-	-	-	8	22	3	2	0	0	10,6	2,6	Curved Up
P. tarda	ZMBN U-4224	-	Universitetsmuseet i Bergen	Sognefjorden	Norway	-	-	-	5,5	15,9	2	2	0	0	8,1	2,1	Straight
P. tarda	ZMBN U-4326	-	Universitetsmuseet i Bergen	Sognefjorden	Norway	-	-	-	7,5	19,4	2	2	0	0	8,7	2,4	Curved Up
P. tarda	131115-1	ZMBN U-4154	Universitetsmuseet i Bergen	Sognefjorden	Norway	-	-	-	7	19,6	4	2	0	1	9	2,3	Curved Up
P. tarda	ZMBN U - 44290	-	Universitetsmuseet i Bergen	Mangersfjorden	Norway	-	-	05.06.19 19	6,5	18	2	4	1	0	9,2	2,4	Curved Up
P. tarda	ZMBN U - 57933	-	Universitetsmuseet i Bergen	Raunefjord	Norway	-	-	1964	4,5	13,5	2	1	0	0	6,6	1,7	Curved Up
P. tarda	ZMBN U - 25369	-	Universitetsmuseet i Bergen	Mangersfjorden	Norway	-	-	28.11.19 19	5,5	18,2	1	1	1	0	8,5	2,3	Straight

Species	ID	Split From	Collect institute	Location	Country	Station	Latitude/Longitude (DMS)	Date	Length cm (telson to rostrum)	Length Carapace mm (dorsal side to eye socket)	Spikes on Basis Left 2nd Pereiopod	Spikes on Basis Right 2nd Pereiopod	Spikes on Left Ischium 2P	Spikes on Right Ischium 2P	Lateral Length Scaphocerite	Width Scaphocerite	Rostrum
P. tarda	ZMBN U - 57699	-	Universitetsmuseet i Bergen	Sørfjorden - Hardangerfjorden	Norway	-	60°21'N 6°39'30''E	20.08.1965	11,5	33	4	1	0	0	14,4	4	Curved Up
P. tarda	ZMBN U - 57699	-	Universitetsmuseet i Bergen	Sørfjorden - Hardangerfjorden	Norway	-	60°21'N 6°39'30''E	20.08.1965	14	38,2	2	3	0	0	18,2	5	Straight
P. tarda	ZMBN U - 57699	-	Universitetsmuseet i Bergen	Sørfjorden - Hardangerfjorden	Norway	-	60°21'N 6°39'30''E	20.08.1965	12	33,3	4	3	1	0	14,7	4	Curved Up
P. tarda	ZMBN U - 60848	-	Universitetsmuseet i Bergen	Korsfjorden	Norway	Biologisk stasjon 74/67	-	23.02.1967	12	33,8	3	3	0	0	15,7	4,2	NA
P. tarda	ZMBN U - 35901	-	Universitetsmuseet i Bergen	Torsken, Manger	Norway	-	-	07.1930	11	32,3	5	6	0	1	15,1	4	Curved Up
P. tarda	ZMBN U - 7029	-	Universitetsmuseet i Bergen	-	Norway	-	-	09.09.1901	14,5	45,6	2	3	0	1	19,5	5,2	Curved Down
P. tarda	ZMBN U - 57698	-	Universitetsmuseet i Bergen	Eidfjorden - Hardangerfjorden	Norway	Z 8-65	-	19.08.1965	12	33	3	2	0	0	15,1	3,9	Straight
P. tarda	ZMBN U - 57697	-	Universitetsmuseet i Bergen	Sørfjorden - Hardangerfjorden	Norway	Z 6-64	60°20'30''N 6°38'40''E	20.08.1964	11,5	32,8	4	3	0	0	14,6	3,8	Straight
P. tarda	ZMBN U - 24745	-	Universitetsmuseet i Bergen	Mangersfjorden	Norway	-	-	05.06.1919	9,5	31	3	3	0	0	15	4,2	Straight
P. tarda	ZMBN U - 7027	-	Universitetsmuseet i Bergen	North Sea	Norway	"M.S." 1901 st. 101	58°10'N 5°5'E	09.09.1901	13	37,9	4	3	0	1	16,7	4,5	Straight
P. tarda	ZMBN U - 7027	-	Universitetsmuseet i Bergen	North Sea	Norway	"M.S." 1901 st. 101	58°10'N 5°5'E	09.09.1901	9,5	29,1	1	1	0	0	13	3,5	Curved Down
P. tarda	ZMBN U - 7027	-	Universitetsmuseet i Bergen	North Sea	Norway	"M.S." 1901 st. 101	58°10'N 5°5'E	09.09.1901	16	46,4	4	3	0	1	20,4	5,1	Curved Down
P. tarda	ZMBN U - 7027	-	Universitetsmuseet i Bergen	North Sea	Norway	"M.S." 1901 st. 101	58°10'N 5°5'E	09.09.1901	14	41,3	3	2	0	1	18,5	5,1	Curved Down
P. tarda	ZMBN U - 14333	-	Universitetsmuseet i Bergen	Skagerak	Norway	"M.S.", Skagerak	-	-	7	19,9	3	3	0	0	9,9	2,5	Straight
P. tarda	ZMBN U - 14333	-	Universitetsmuseet i Bergen	Skagerak	Norway	"M.S.", Skagerak	-	-	8	23,2	5	4	0	0	10,5	2,8	Curved Up
P. tarda	ZMBN U - 14333	-	Universitetsmuseet i Bergen	Skagerak	Norway	"M.S.", Skagerak	-	-	8,5	23,4	4	3	1	0	11,6	3	Curved Up
P. tarda	ZMBN U - 14333	-	Universitetsmuseet i Bergen	Skagerak	Norway	"M.S.", Skagerak	-	-	NA	NA	2	2	0	0	NA	NA	Curved up
P. tarda	ZMBN U - 14333	-	Universitetsmuseet i Bergen	Skagerak	Norway	"M.S.", Skagerak	-	-	NA	NA	2	2	0	0	NA	NA	Curved Up
P. tarda	ZMBN U - 14333	-	Universitetsmuseet i Bergen	Skagerak	Norway	"M.S.", Skagerak	-	-	NA	NA	0	0	0	0	NA	NA	Curved Up
P. tarda	ZMBN U - 14333	-	Universitetsmuseet i Bergen	Skagerak	Norway	"M.S.", Skagerak	-	-	NA	NA	0	0	0	0	NA	NA	Curved Up

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P. tarda	ZMBN U - 10938	-	Universitetsmuseet i Bergen	West Coast of Norland	Norway	"M.S." 1907 st. 82	65°27'N 11°48'E	29.10. 1907	7	19,8	3	4	1	1	10,3	2,6	Straight
P. tarda	ZMBN U - 10938	-	Universitetsmuseet i Bergen	West Coast of Norland	Norway	"M.S." 1907 st. 82	65°27'N 11°48'E	29.10. 1907	7	19,1	3	4	1	1	10	2,5	Curved Up
P. tarda	ZMBN U - 10938	-	Universitetsmuseet i Bergen	West Coast of Norland	Norway	"M.S." 1907 st. 82	65°27'N 11°48'E	29.10. 1907	NA	NA	0	0	0	0	NA	NA	Curved Up
P. tarda	ZMBN U - 10938	-	Universitetsmuseet i Bergen	West Coast of Norland	Norway	"M.S." 1907 st. 82	65°27'N 11°48'E	29.10. 1907	NA	NA	0	0	0	0	NA	NA	Curved Up
P. tarda	ZMBN U - 10938	-	Universitetsmuseet i Bergen	West Coast of Norland	Norway	"M.S." 1907 st. 82	65°27'N 11°48'E	29.10. 1907	NA	NA	0	0	0	0	NA	NA	Curved Up
P. tarda	ZMBN U - 10938	-	Universitetsmuseet i Bergen	West Coast of Norland	Norway	"M.S." 1907 st. 82	65°27'N 11°48'E	29.10. 1907	NA	NA	0	0	0	0	NA	NA	Curved Up
P. tarda	ZMBN U - 10938	-	Universitetsmuseet i Bergen	West Coast of Norland	Norway	"M.S." 1907 st. 82	65°27'N 11°48'E	29.10. 1907	NA	NA	0	0	0	0	NA	NA	Curved Up
P. tarda	ZMBN U - 10938	-	Universitetsmuseet i Bergen	West Coast of Norland	Norway	"M.S." 1907 st. 82	65°27'N 11°48'E	29.10. 1907	NA	NA	2	1	1	1	NA	NA	Curved Up
P. tarda	1988-0184 (Acq 1961-36)	-	Canadian Museum of Science	Green Bank Gully - New Foundland	Canada East	-	44°57' N 54°55'48'' W	10.10. 1957	13,5	38,3	1	2	0	0	17,6	4,9	NA
P. tarda	2004-3044 (Acq 1983-144)	-	Canadian Museum of Science	Labrador Shelf	Canada East	-	55°0' N 55°30' W	04.11. 1977	13,5	40	1	2	0	0	17	5,1	NA
P. tarda	1988-0187 (Acq 1963-242)	-	Canadian Museum of Science	New Foundland	Canada East	-	51°28'12'' N 53°44'24'' W	31.05. 1963	12	34,4	6	7	0	1	15,7	4,5	Curved Down
P. tarda	151015-1	2015-0011 (Acq: A2015.0035)	Canadian Museum of Science	Baffin Bay	Canada East	-	61°58'48'' N 60°43'48'' W	15.10. 2014	12	38,6	4	4	0	0	16,9	4,7	Straight
P. tarda	151015-2	2015-0011 (Acq: A2015.0035)	Canadian Museum of Science	Baffin Bay	Canada East	-	61°58'48'' N 60°43'48'' W	15.10. 2014	14,5	40,4	1	2	0	0	18,4	5,1	Curved Down
P. tarda	151015-3	2015-0011 (Acq: A2015.0035)	Canadian Museum of Science	Baffin Bay	Canada East	-	61°58'48'' N 60°43'48'' W	15.10. 2014	20	58,7	2	1	0	0	25,6	6,6	Curved Down
P. tarda	009-00057-012	-	Royal BC Museum	Vancouver Island	Canada	-	-	1999	17,5	54,6	8	7	1	1	22,8	6,4	Curved Down

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P. tarda	009-00057-012	-	Royal BC Museum	Vancouver Island	Canada	-	-	1999	10	33,7	0	0	0	0	13,5	3,9	Curved Down
P. tarda	009-00057-012	-	Royal BC Museum	Vancouver Island	Canada			1999	18,5	56,5	6	4	1	2	24	7	Curved Down
P. tarda	009-00057-012	-	Royal BC Museum	Vancouver Island	Canada	-	-	1999	16	49	8	7	1	0	20,5	5,6	Curved Down
P. tarda	009-00057-012	-	Royal BC Museum	Vancouver Island	Canada	-	-	1999	18	55,5	4	5	1	0	23	6,6	Curved Down
P. tarda	291015-3	010-0262-006	Royal BC Museum	Father Charles Canyon	Canada	-	48°35.892' N 126°54.538' W	04.09.2001	14,5	42,8	0	0	0	0	17,7	5,7	Straight
P. tarda	010-00262-006	-	Royal BC Museum	Father Charles Canyon	Canada	-	48°35.892' N 126°54.538' W	04.09.2001	6,5	19,5	0	0	0	0	9,2	2,9	Straight
P. tarda	010-00262-006	-	Royal BC Museum	Father Charles Canyon	Canada	-	48°35.892' N 126°54.538' W	04.09.2001	9,5	28,6	0	0	0	0	11,9	4,1	Curved up
P. tarda	010-00262-006	-	Royal BC Museum	Father Charles Canyon	Canada	-	48°35.892' N 126°54.538' W	04.09.2001	6,5	20,5	0	0	0	0	9	2,9	Curved up
P. tarda	010-00247-011	-	Royal BC Museum	Vancouver Island	Canada	-	48°35.892' N 126°54.538' W	04.09.2001	17,5	55	5	4	1	1	21,2	6,2	Straight
P. tarda	291015-1	010-00260-004	Royal BC Museum	Vancouver Island	Canada	-	48°19.947' N 126°23.746' W	03.09.2001	19,5	57,1	4	6	1	1	23	6,7	Straight
P. tarda	010-00260-004	-	Royal BC Museum	Vancouver Island	Canada	-	48°19.947' N 126°23.746' W	03.09.2001	6	16,8	3	3	1	1	7,8	2	Curved up
P. tarda	013-00043-002	-	Royal BC Museum	Vancouver Island	Canada	-	48°19.947' N 126°23.746' W	03.09.2001	14,5	46,8	4	4	1	0	19,5	5,3	Curved up
P. tarda	013-00043-002	-	Royal BC Museum	Vancouver Island	Canada	-	48°19.947' N 126°23.746' W	07.10.2006	14,5	45,5	0	0	0	0	16,5	6	Straight
P. tarda	013-00043-002	-	Royal BC Museum	Vancouver Island	Canada	-	48°19.947' N 126°23.746' W	07.10.2006	17,3	52,2	4	5	1	1	22,8	6,2	Straight
P. tarda	013-00043-002	-	Royal BC Museum	Vancouver Island	Canada	-	48°19.947' N 126°23.746' W	07.10.2006	14	43	0	0	0	0	16,4	5,8	Curved up
P. tarda	013-00043-002	-	Royal BC Museum	Vancouver Island	Canada	-	48°19.947' N 126°23.746' W	07.10.2006	11,5	34,4	5	7	1	1	14,9	4,3	Curved up
P. tarda	010-00228-006	-	Royal BC Museum	Gowgaia Bay	Canada	-	52°20.074' N 131°51.680' W	04.09.2006	15	45,3	0	0	0	0	17,9	6	Curved up
P. tarda	010-00228-006	-	Royal BC Museum	Gowgaia Bay	Canada	-	52°20.074' N 131°51.680' W	04.09.2006	14,5	44,2	0	0	0	0	16,9	5,6	Curved up
P. tarda	010-00228-006	-	Royal BC Museum	Gowgaia Bay	Canada	-	52°20.074' N 131°51.680' W	04.09.2006	7,5	23,6	5	4	1	1	10,1	3	Curved up
P. tarda	010-00228-006	-	Royal BC Museum	Gowgaia Bay	Canada	-	52°20.074' N 131°51.680' W	04.09.2006	12,5	36	8	6	1	1	15,5	4,6	Curved up

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P. tarda	010-00228-006	-	Royal BC Museum	Gowgaia Bay	Canada	-	52°20.074' N 131°51.680' W	04.09.2006	15	47,4	0	0	0	0	17,5	5,7	Curved up
P. tarda	010-00299-008	-	Royal BC Museum	Graham Island	Canada	-	54°05.027' N 134°07.116' W	02.02.2002	13,5	46,6	0	0	0	0	17,5	6,2	Straight
P. tarda	010-00299-008	-	Royal BC Museum	Graham Island	Canada	-	54°05.027' N 134°07.116' W	02.02.2002	11	35,6	0	0	0	0	14,3	4,6	Straight
P. tarda	010-00299-008	-	Royal BC Museum	Graham Island	Canada	-	54°05.027' N 134°07.116' W	02.02.2002	14,5	47,2	0	0	0	0	18,6	6	Straight
P. tarda	010-00299-008	-	Royal BC Museum	Graham Island	Canada	-	54°05.027' N 134°07.116' W	02.02.2002	13	45	0	0	0	0	16,8	5,5	Straight
P. tarda	010-00299-008	-	Royal BC Museum	Graham Island	Canada	-	54°05.027' N 134°07.116' W	02.02.2002	11,5	36,7	0	0	0	0	14,8	5	Curved up
P. tarda	010-00299-008	-	Royal BC Museum	Graham Island	Canada	-	54°05.027' N 134°07.116' W	02.02.2002	9,5	30,3	0	0	0	0	13,1	4,1	Straight
P. tarda	010-00299-008	-	Royal BC Museum	Graham Island	Canada	-	54°05.027' N 134°07.116' W	02.02.2002	12,5	38,7	0	0	0	0	16,2	5,3	Curved up
P. tarda	010-00299-008	-	Royal BC Museum	Graham Island	Canada	-	54°05.027' N 134°07.116' W	02.02.2002	8,5	27,6	0	0	0	0	12	3,8	Curved up
P. tarda	979-11252-8	-	Royal BC Museum	Ocean Station Papa	Canada	-	50° N 145° W	31.08.1979	6	16,7	4	4	1	1	8,2	2,3	Curved up
P. tarda	979-11252-8	-	Royal BC Museum	Ocean Station Papa	Canada	-	50° N 145° W	31.08.1979	3,5	11,3	0	0	0	0	5,6	2	Curved up
P. tarda	979-11252-8	-	Royal BC Museum	Ocean Station Papa	Canada	-	50° N 145° W	31.08.1979	2,5	9,5	0	0	0	0	4,4	1,4	Curved up
P. tarda	291015-4	979-11252-8	Royal BC Museum	Ocean Station Papa	Canada	-	50° N 145° W	31.08.1979	6	18,3	3	3	NA	NA	9,7	2,6	Curved up
P. tarda	ZMBN U - 84137	-	Universitetsmuseet i Bergen	Mid Atlantic Ridge		MAR-ECO 62-380-1162	51°55'N 30° 25'W	20.07.2004	7,5	22,6	0	0	0	0	10,2	2,8	Curved Up
P. tarda	ZMBN U - 84137	-	Universitetsmuseet i Bergen	Mid Atlantic Ridge		MAR-ECO 62-380-1162	51°55'N 30° 25'W	20.07.2004	8	25,2	0	0	0	0	11,5	3	Curved Up

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P. tarda	ZMBN U - 84087	-	Universitetsmuseet i Bergen	Mid Atlantic Ridge		MAR-ECO 70-385-1167	52°58'N 34° 52'W	26.07.2004	6,5	20	0	0	0	0	9,2	2,6	Curved Up
P. tarda	ZMBN U - 84087	-	Universitetsmuseet i Bergen	Mid Atlantic Ridge		MAR-ECO70-385-1167	52°58'N 34° 52'W	26.07.2004	9	26,8	0	0	0	0	13,2	3,5	Curved Up
P. tarda	ZMBN U - 84087	-	Universitetsmuseet i Bergen	Mid Atlantic Ridge		MAR-ECO 70-385-1167	52°58'N 34° 52'W	26.07.2004	8	24,4	0	0	0	0	10,6	3	Curved Up
P. tarda	211015-2	ZMBN U - 84087	Universitetsmuseet i Bergen	Mid Atlantic Ridge		MAR-ECO 70-385-1167	52°58'N 34° 52'W	26.07.2004	10	32,1	0	0	0	0	14,6	4	Curved Up
P. tarda	211015-1	ZMBN U - 84087	Universitetsmuseet i Bergen	Mid Atlantic Ridge		MAR-ECO 70-385-1167	52°58'N 34° 52'W	26.07.2004	8,5	26,6	0	0	0	0	11,7	3,2	Curved Up
P. tarda	051215-1	ZMBN U - 84138	Universitetsmuseet i Bergen	Mid Atlantic Ridge		MAR-ECO 53-375-1157	49°51'N 29° 37'W	15.07.2004	10,5	30,9	3	1	0	0	14,2	4,1	Straight
P. tarda	051215-2	ZMBN U - 84138	Universitetsmuseet i Bergen	Mid Atlantic Ridge		MAR-ECO 53-375-1157	49°51'N 29° 37'W	15.07.2004	9,5	28,9	3	5	0	0	13,6	3,5	Straight
P. tarda	051215-3	ZMBN U - 84138	Universitetsmuseet i Bergen	Mid Atlantic Ridge		MAR-ECO 53-375-1157	49°51'N 29° 37'W	15.07.2004	11	31,2	5	5	1	1	14,7	3,9	Straight
P. tarda	051215-4	ZMBN U - 84138	Universitetsmuseet i Bergen	Mid Atlantic Ridge		MAR-ECO 53-375-1157	49°51'N 29° 37'W	15.07.2004	10	28,4	3	4	1	1	14,1	3,8	Straight
P. tarda	051215-5	ZMBN U - 84138	Universitetsmuseet i Bergen	Mid Atlantic Ridge		MAR-ECO 53-375-1157	49°51'N 29° 37'W	15.07.2004	10,5	32,4	5	5	0	NA	15	4	Straight
P. tarda	051215-6	ZMBN U - 84138	Universitetsmuseet i Bergen	Mid Atlantic Ridge		MAR-ECO 53-375-1157	49°51'N 29° 37'W	15.07.2004	8,5	26,2	4	5	0	0	12	3	Straight
P. tarda	051215-7	ZMBN U - 84138	Universitetsmuseet i Bergen	Mid Atlantic Ridge		MAR-ECO 53-375-1157	49°51'N 29° 37'W	15.07.2004	9,5	27,8	3	4	1	0	12,5	3,3	Curved Up
P. tarda	ZMBN U - 84088	-	Universitetsmuseet i Bergen	Mid Atlantic Ridge		MAR-ECO 72-386-1168	53°16'N 35°31'W	27.07.2004	15	45,5	0	0	0	0	18,7	5,6	Curved Up
P. tarda	051215-8	ZMBN U - 84088	Universitetsmuseet i Bergen	Mid Atlantic Ridge		MAR-ECO 72-386-1168	53°16'N 35°31'W	27.07.2004	15	43,5	0	0	0	0	19	5,6	Curved Up
P. tarda	051215-9	ZMBN U - 84088	Universitetsmuseet i Bergen	Mid Atlantic Ridge		MAR-ECO 72-386-1168	53°16'N 35°31'W	27.07.2004	12	36,4	0	0	0	0	16,2	4,2	Curved Up
P. tarda	051215-10	ZMBN U - 84088	Universitetsmuseet i Bergen	Mid Atlantic Ridge		MAR-ECO 72-386-1168	53°16'N 35°31'W	27.07.2004	11,5	35,7	0	0	0	0	16,9	4,4	Curved Up

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P. tarda	051215-11	ZMBN U - 84088	Universitetsmuseet i Bergen	Mid Atlantic Ridge		MAR-ECO 72-386-1168	53°16'N 35°31'W	27.07.2004	11,5	34,4	0	0	0	0	15,4	4,4	Curved Up
P. tarda	211015-9	ZMBN U - 84138	Universitetsmuseet i Bergen	Mid Atlantic Ridge		MAR-ECO 53-375-1157	49°51'N 29° 37'W	15.07.2004	11,5	34	6	6	0	0	15,5	4	Straight
P. tarda	211015-7	ZMBN U - 84138	Universitetsmuseet i Bergen	Mid Atlantic Ridge		MAR-ECO53-375-1157	49°51'N 29° 37'W	15.07.2004	12	34,3	5	4	1	1	15,3	4,4	Straight
P. tarda	211015-8	ZMBN U - 84138	Universitetsmuseet i Bergen	Mid Atlantic Ridge		MAR-ECO53-375-1157	49°51'N 29° 37'W	15.07.2004	11	32,3	2	2	0	0	15,5	4	Straight
P. tarda	211015-6	ZMBN U - 84138	Universitetsmuseet i Bergen	Mid Atlantic Ridge		MAR-ECO 53-375-1157	49°51'N 29° 37'W	15.07.2004	10,5	29,7	3	5	0	0	13,9	3,8	Curved Up
P. tarda	LS 8900	-	Universitetsmuseet i Bergen	Mid Atlantic Ridge		LS-7-332	57°5'6.72'' N 31°21'37.08'' W	13.6.2004	13,5	40,6	0	2	0	0	17,5	4,3	Straight
P. tarda	LS 201015-2	LS 12300	Universitetsmuseet i Bergen	Mid Atlantic Ridge		LS-26-354	48°0'10.8'' N 29°34'13.8'' W	25.6.2004	11	30,5	5	4	0	0	14,5	3,7	Straight
P. tarda	LS 201015-3	LS 12300	Universitetsmuseet i Bergen	Mid Atlantic Ridge		LS-26-355	48°0'10.8'' N 29°34'13.8'' W	25.6.2004	10,5	29,9	3	3	1	0	13,1	3,4	Curved Down
P. tarda	NMSZ - 1996.3.132	-	National Museum of Scotland	West of Scotland		Station 18	56°27' N 09°17' W	21.04.1985	12	32,1	5	4	1	1	16,6	4	Curved Up
P. tarda	NMSZ - 1978.59.123	-	National Museum of Scotland	Bay of Biscay		Station S78/27	47°21' N 8°19' W	16.05.1978	NA	NA	0	0	0	0	NA	NA	Curved Up
P. tarda	NMSZ - 1978.51.94		National Museum of Scotland	Bay of Biscay		Station S77/13	47°12' N 7°37' W	06.1977	NA	NA	1	0	1	1	NA	NA	Curved Up
P. tarda	NMSZ - 1955.63	-	National Museum of Scotland	West of Scotland		-	58°43' N 09°45' W	23.08.1910	8	25	2	2	1	1	12,7	3,1	Curved Up
P. tarda	NMSZ - 1908.175.3	-	National Museum of Scotland	South West of Ireland		Stn S.R. 505	50°39' N 11°14' W	12.09.1907	6,5	17,7	2	1	0	1	8,8	2,3	Curved Up
P. tarda	A55	-	National Museum of Scotland	West of Ireland		-	55°01' N 11°34' W	02.11.1973	NA	NA	2	3	1	0	NA	NA	Curved Up
P. tarda	A55	-	National Museum of Scotland	West of Ireland		-	55°01' N 11°34' W	02.11.1973	NA	NA	0	0	0	0	NA	NA	Spiky Curved Up
P. tarda	A55	-	National Museum of Scotland	West of Ireland		-	55°01' N 11°34' W	02.11.1973	NA	NA	0	0	0	0	NA	NA	Spiky Curved Up

Species	ID	Split From	Collect institute	Location	Country	Station	Latitude/Longitude (DMS)	Date	Length cm (telson to rostrum)	Length Carapace mm (dorsal side to eye socket)	Spikes on Basis Left 2nd Pereiopod	Spikes on Basis Right spesimen2nd Pereiopod	Spikes on Left Ischium 2P	Spikes on Right Ischium 2P	Lateral Length Scaphocerite	Width Scaphocerite	Rostrum
P. tarda	A55	-	National Museum of Scotland	West of Ireland		-	55°01' N 11°34' W	02.11.1973	NA	NA	0	0	0	0	NA	NA	Spiky Curved Up
P. tarda	A70	-	National Museum of Scotland	Azores		-	40° N 30° W	29.04.1974	NA	NA	0	0	0	0	NA	NA	Spiky Curved Up
P. tarda	A70	-	National Museum of Scotland	Azores		-	40° N 30° W	29.04.1974	NA	NA	0	0	0	0	NA	NA	Spiky Curved Up
P. tarda	A70	-	National Museum of Scotland	Azores		-	40° N 30° W	29.04.1974	NA	NA	0	0	0	0	NA	NA	Spiky Curved Up
P. tarda	A117	-	National Museum of Scotland	South West of Ireland		-	50°03' N 12°03' W	15.11.1975	NA	NA	0	0	0	0	NA	NA	Spiky Curved Up
P. tarda	A117	-	National Museum of Scotland	South West of Ireland		-	50°03' N 12°03' W	15.11.1975	NA	NA	0	0	0	0	NA	NA	Spiky Curved Up
P. tarda	A135	-	National Museum of Scotland	South West of Portugal		-	36°16' N 12°15' W	20.10.1975	NA	NA	0	0	0	0	NA	NA	Spiky Curved Up
P. tarda	DQ882139	-	Biodiversity Institute of Ontario	British Columbia	Canada	-	-	13.04.2003	10,5	34,5	8	7	1	1	13,9	4	Curved Up
P. tarda	DQ882138	-	Biodiversity Institute of Ontario	British Columbia	Canada	-	-	13.04.2003	17	51	9	7	2	1	20	10	NA
Pasiphaea tarda (type)	ZMUR CRU-9387	-	Universitetsmuseet i København	South of Greenland	Denmark	-	-	1842	13	37,2	4	3	0	0	NA	4,5	NA
P. tarda	-	-	Universitetsmuseet i København	Skagerak	Norway	-	57°52' N 8°1' E	08.07.1912	11,5	27,8	2	1	0	0	18,8	3,2	Curved Down
P. tarda	-	-	Universitetsmuseet i København	-	-	-	-	28.051907	12,5	34	3	3	1	1	14,9	4,1	Straight
P. tarda	-	-	Universitetsmuseet i København	-	-	-	-	28.051908	8	24,5	1	1	0	0	11,3	2,7	Curved Up
P. tarda	-	-	Universitetsmuseet i København	-	-	-	-	28.051909	12,5	34,7	3	4	1	0	15,1	4,2	Curved Down
P. tarda	-	-	Universitetsmuseet i København	Skagerak	Norway	-	58°5' N 8°24' E	23.06.1907	11,5	34,2	3	3	1	0	15,7	4,1	Straight
P. tarda	-	-	Universitetsmuseet i København	Skagerak	Norway	-	58°20' N 9°0' E	30.06.1907	13	39,7	4	4	0	0	18,1	4,5	Straight
P. tarda	-	-	Universitetsmuseet i København	-	-	-	-	14.10.1904	13,5	42,2	2	4	0	1	18,8	4,8	NA
P. tarda	-	-	Universitetsmuseet i København	-	-	-	-	14.10.1904	6	16,6	2	2	0	0	7,8	2,1	Curved Up
P. tarda	-	-	Universitetsmuseet i København	Skagerak	-	-	-	-	8,5	24,2	2	2	0	0	11,5	2,9	Curved Up
P. tarda	-	-	Universitetsmuseet i København	Skagerak	-	-	-	-	9	24,4	3	2	0	0	11,4	2,6	Straight

Species	Collect institute	Location	Country	Latitude/Longitude (DMS)	Date	Length cm (telson to rostrum)	Length Carapace mm (dorsal side to eye socket)	Spikes on Basis Left 2nd Pereiopod	Spikes on Basis Right 2nd Pereiopod	Spikes on Left Ischium 2P	Spikes on Right Ischium 2P	Lateral Length Scaphocerite	Width Scaphocerite	Rostrum
P. tarda	Universitetsmuseet i København	Skagerak	-	-	-	9,5	26	3	4	0	0	12,1	3,2	Curved Up
P. tarda	Universitetsmuseet i København	Skagerak	-	-	-	9	23,6	3	3	0	0	11,2	3	Curved Up
P. tarda	Universitetsmuseet i København	Skagerak	Norway	58°16' N 9°37' E	03.04.1965	9,5	26,5	1	1	0	0	11,1	3,5	Curved Up
P. tarda	Universitetsmuseet i København	Skagerak	Norway	58°16' N 9°37' E	03.04.1965	14,5	45	4	4	0	1	18	5	NA
P. tarda	Universitetsmuseet i København	Skagerak	Norway	58°16' N 9°37' E	03.04.1965	11	32,1	2	2	0	0	14,3	3,8	Straight
P. tarda	Universitetsmuseet i København	Skagerak	Norway	58°16' N 9°37' E	03.04.1965	14	43	1	3	0	0	18,6	5	Curved Down
P. tarda	Universitetsmuseet i København	Skagerak	Norway	58°16' N 9°37' E	03.04.1965	13	37,7	4	4	0	1	17	4,3	Straight
P. tarda	Universitetsmuseet i København	Skagerak	Norway	58°16' N 9°37' E	03.04.1965	13,5	41	2	2	0	0	18,8	4,8	Curved Down
P. tarda	Universitetsmuseet i København	Skagerak	Norway	58°3' N 9°20' E	17.07.1912	12	36,4	1	1	0	0	17	4,5	Straight
P. tarda	Universitetsmuseet i København	Skagerak	Norway	58°3' N 9°20' E	17.07.1912	8	22,4	3	3	0	0	10,7	2,8	Curved Up
P. tarda	Universitetsmuseet i København	Celtic Sea	Ireland	49°23' N 12°13' W	09.06.1906	NA	23,7	2	1	1	1	10,4	2,8	Curved Up
P. tarda	Universitetsmuseet i København	Celtic Sea	Ireland	49°23' N 12°13' W	09.06.1906	7,5	21	2	1	0	0	10,1	2,8	Curved Up
P. tarda	Universitetsmuseet i København	Kvanefjord - Greenland	Denmark	-	-	13	37	5	3	0	0	16,5	4,7	Curved Down
P. tarda	Universitetsmuseet i København	West of Greenland	Denmark	64°14' N 55°55' W	02.06.1909	10	28,9	1	2	0	0	13	3,5	NA
P. tarda	Universitetsmuseet i København	Greenland	Denmark	-	1953	12	33,1	5	5	1	1	14,7	4	Curved Up
P. tarda	Universitetsmuseet i København	Greenland	Denmark	-	1953	12	33,6	2	2	0	0	15	3,9	Straight
P. tarda	Universitetsmuseet i København	Greenland	Denmark	-	1953	12	36,1	2	4	0	0	15,3	4	Straight

Species	ID	Collect institute	Location	Country	Date	Length cm (telson to rostrum)	Length Carapace mm (dorsal side to eye socket)	Spikes on Basis Left 2nd Pereiopod	Spikes on Basis Right 2nd Pereiopod	Spikes on Left Ischium 2P	Spikes on Right Ischium 2P	Lateral Length Scaphocerite	Width Scaphocerite	Rostrum
Pasiphaea princeps (type)	5473	Smithsonian Museum Washington DC	NE Coast U.S.A	U.S.A.	1883	23,5	71,6	0	0	0	0	28	8,6	Curved Up
P. princeps	10709	Smithsonian Museum Washington DC	NE Coast U.S.A	U.S.A.	14.07.1885	4,5	12,3	0	0	0	0	5,7	1,7	Curved Up
P. princeps	10710	Smithsonian Museum Washington DC	SE Coast Canada	Canada	23.06.1885	3,5	10,6	0	0	0	0	4,8	1,3	Curved Up
P. princeps	10710	Smithsonian Museum Washington DC	SE Coast Canada	Canada	23.06.1885	3,5	9,7	0	0	0	0	5,4	1,6	Curved Up
P. princeps	31454	Smithsonian Museum Washington DC	NE Coast U.S.A	U.S.A.	-	5,5	14,9	1	2	0	0	7,9	2,1	Curved Up

Appendix 2 – Thermo cycler programs

Table A2.1. Barcoding program applied in amplification of the CO1 gene via the PCR method.

	Temperature (°C)	Time (minutes)	Cycles
	Barcoding		
Start	94	05:00	x1
	94	00:45	
	45	00:30	
	72	01:00	x5
	94	00:45	
	50	00:30	
	72	01:00	x31
	72	10:00	x1
End	6	forever	

Table A2.2. EXOSAP program applied to remove leftover primers and dNTPs.

	Temperature (°C)	Time (minutes)	Cycles
	EXOSAP		
Start	37	30;00	x1
	87	15;00	x1
End	4	forever	

Table A2.3. SEQ program applied to prepare the PCR products for sequencing in accordance with the BigDye® version 3.1 sequencing protocol

	Temperature (°C)	Time (minutes)	Cycles
	SEQ		
Start	96	05:00	x1
	96	00:10	
	50	00:05	
	60	04:00	x25
End	4	forever	

Appendix 3 – ML model parameters

Table A3.1. Model parameters generated by using the inbuilt "Find best DNA model (ML)" function in MEGA (MEGA, version 6.06, 2015), ordered by descending overall scores.

Model	#Param	BIC	AICc	lnL	Invariant	Gamma	R	Freq A	Freq T	Freq C	Freq G
T92+G+I	145	7783,26	6525,19	-3117,11	0,48	1,80	3,09	0,31	0,31	0,19	0,19
T92+I	144	7784,53	6535,13	-3123,08	0,54	n/a	2,80	0,31	0,31	0,19	0,19
T92+G	144	7817,24	6567,84	-3139,44	n/a	0,68	2,63	0,31	0,31	0,19	0,19
GTR+I	150	7820,97	6519,56	-3109,26	0,54	n/a	2,75	0,26	0,35	0,20	0,19
GTR+G+I	151	7825,44	6515,36	-3106,15	0,47	1,99	2,72	0,26	0,35	0,20	0,19
GTR+G	150	7856,46	6555,05	-3127,00	n/a	0,71	2,39	0,26	0,35	0,20	0,19
K2+G+I	144	7863,85	6614,45	-3162,74	0,45	1,74	2,71	0,25	0,25	0,25	0,25
K2+I	143	7864,88	6624,15	-3168,60	0,54	n/a	2,52	0,25	0,25	0,25	0,25
K2+G	143	7889,68	6648,95	-3181,00	n/a	0,72	2,44	0,25	0,25	0,25	0,25
T92	143	8076,40	6835,67	-3274,36	n/a	n/a	2,11	0,31	0,31	0,19	0,19
GTR	149	8098,00	6805,26	-3253,11	n/a	n/a	1,91	0,26	0,35	0,20	0,19
JC+I	142	8112,03	6879,97	-3297,52	0,52	n/a	0,50	0,25	0,25	0,25	0,25
JC+G+I	143	8116,12	6875,40	-3294,22	0,46	2,73	0,50	0,25	0,25	0,25	0,25
K2	142	8119,20	6887,15	-3301,11	n/a	n/a	2,08	0,25	0,25	0,25	0,25
JC+G	142	8137,60	6905,55	-3310,31	n/a	0,79	0,50	0,25	0,25	0,25	0,25
JC	141	8339,27	7115,89	-3416,48	n/a	n/a	0,50	0,25	0,25	0,25	0,25
HKY+I	146	30551,76	29285,03	-14496,02	0,54	n/a	2,71	0,26	0,35	0,20	0,19
TN93+I	147	30560,36	29284,95	-14494,97	0,54	n/a	2,72	0,26	0,35	0,20	0,19
HKY+G+I	147	30592,77	29317,37	-14511,18	0,00	0,33	3,03	0,26	0,35	0,20	0,19
TN93+G+I	148	30601,92	29317,85	-14510,42	0,43	1,26	3,07	0,26	0,35	0,20	0,19
HKY+G	146	30619,00	29352,26	-14529,64	n/a	0,70	2,56	0,26	0,35	0,20	0,19
TN93+G	147	30627,61	29352,20	-14528,60	n/a	0,71	2,59	0,26	0,35	0,20	0,19
HKY	145	30830,50	29572,43	-14640,73	n/a	n/a	2,14	0,26	0,35	0,20	0,19
TN93	146	30838,10	29571,37	-14639,19	n/a	n/a	2,14	0,26	0,35	0,20	0,19

Table A3.2. Continuation of table A3.1 showing frequencies of nucleotides and the substitution rates for the respective models.

| A=>T	A=>C	A=>G	T=>A	T=>C	T=>G	C=>A	C=>T	C=>G	G=>A	G=>T	G=>C
0,04	0,02	0,15	0,04	0,15	0,02	0,04	0,23	0,02	0,23	0,04	0,02
0,04	0,02	0,14	0,04	0,14	0,02	0,04	0,23	0,02	0,23	0,04	0,02
0,04	0,03	0,14	0,04	0,14	0,03	0,04	0,23	0,03	0,23	0,04	0,03
0,07	0,02	0,12	0,05	0,17	0,02	0,02	0,29	0,01	0,17	0,04	0,01
0,06	0,01	0,12	0,05	0,16	0,02	0,02	0,28	0,03	0,17	0,04	0,03
0,07	0,02	0,12	0,05	0,15	0,02	0,02	0,27	0,03	0,17	0,04	0,03
0,03	0,03	0,18	0,03	0,18	0,03	0,03	0,18	0,03	0,18	0,03	0,03
0,04	0,04	0,18	0,04	0,18	0,04	0,04	0,18	0,04	0,18	0,04	0,04
0,04	0,04	0,18	0,04	0,18	0,04	0,04	0,18	0,04	0,18	0,04	0,04
0,05	0,03	0,13	0,05	0,13	0,03	0,05	0,21	0,03	0,21	0,05	0,03
0,09	0,02	0,12	0,07	0,14	0,02	0,03	0,25	0,03	0,16	0,04	0,03
0,08	0,08	0,08	0,08	0,08	0,08	0,08	0,08	0,08	0,08	0,08	0,08
0,08	0,08	0,08	0,08	0,08	0,08	0,08	0,08	0,08	0,08	0,08	0,08
0,04	0,04	0,17	0,04	0,17	0,04	0,04	0,17	0,04	0,17	0,04	0,04
0,08	0,08	0,08	0,08	0,08	0,08	0,08	0,08	0,08	0,08	0,08	0,08
0,08	0,08	0,08	0,08	0,08	0,08	0,08	0,08	0,08	0,08	0,08	0,08
0,05	0,03	0,14	0,03	0,15	0,02	0,03	0,26	0,02	0,19	0,05	0,03
0,05	0,03	0,12	0,03	0,16	0,02	0,03	0,28	0,02	0,17	0,05	0,03
0,04	0,02	0,14	0,03	0,15	0,02	0,03	0,27	0,02	0,2	0,04	0,02
0,04	0,02	0,12	0,03	0,17	0,02	0,03	0,31	0,02	0,16	0,04	0,02
0,05	0,03	0,14	0,04	0,14	0,03	0,04	0,26	0,03	0,19	0,05	0,03
0,05	0,03	0,12	0,04	0,16	0,03	0,04	0,29	0,03	0,16	0,05	0,03
0,05	0,03	0,13	0,04	0,14	0,03	0,04	0,24	0,03	0,18	0,05	0,03
0,06	0,03	0,11	0,04	0,15	0,03	0,04	0,26	0,03	0,16	0,06	0,03
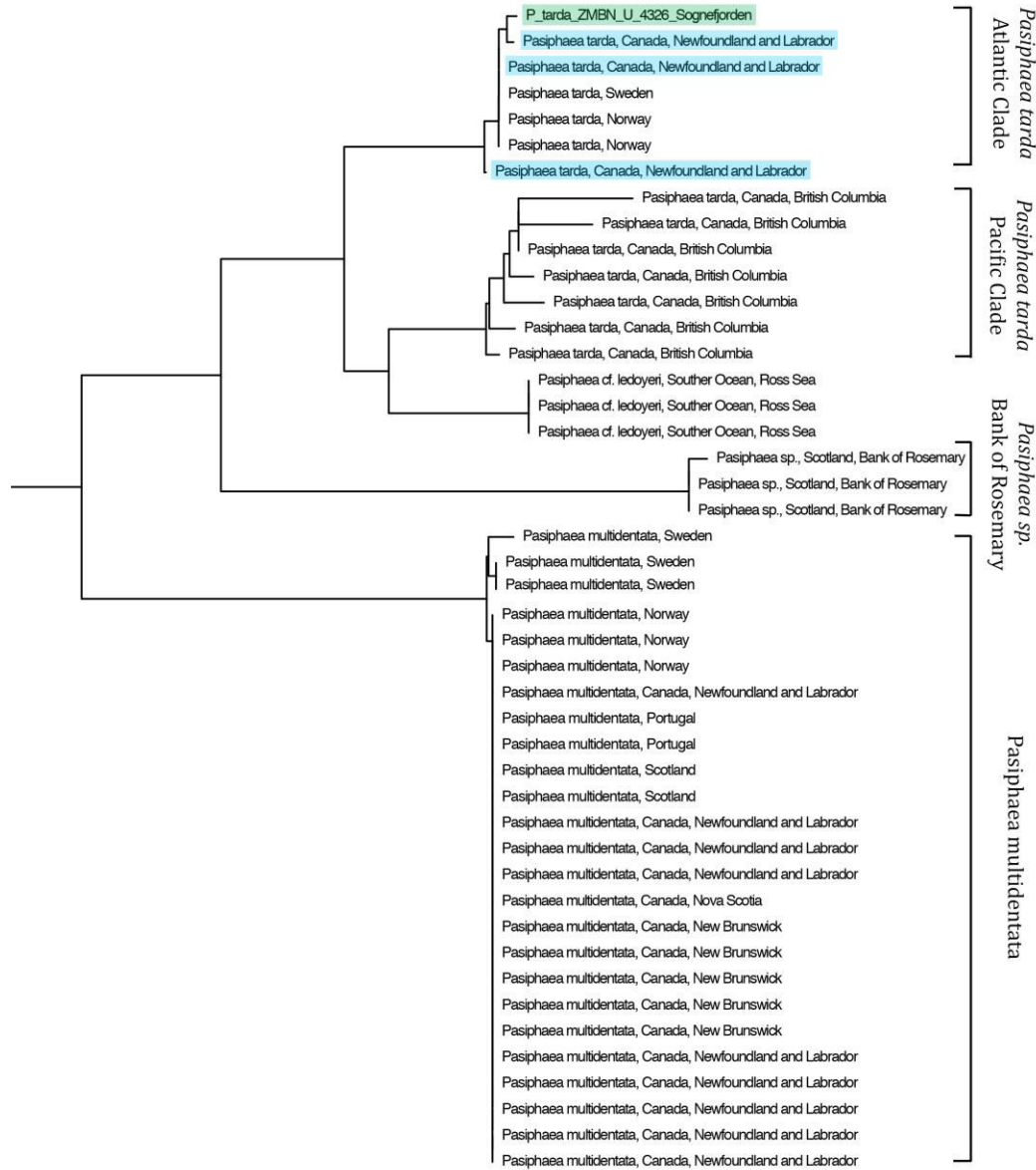
Figure A4.1. NJ tree of the *Pasiphaea* genus consisting of 45 sequences. The tree was constructed utilizing the online tree generator available from boldsystems.org. The tree indicates that sequences of specimens from the northwest Atlantic (marked in blue) are genetically similar to a sequence from the Sognefjord (marked in green) and additional sequences from the northeast Atlantic. The approximate origin of the northwestern Atlantic sequences can be viewed in figure 4.2.1 marked with a yellow dot at location 6.

Appendix 5 - R Scripts

5.1 Analyses comparing populations

#Analysis of "spikes_on_basis_2nd_pereiopod"

```
plot(spikes_on_basis_2nd_pereiopod~location , family="quasipoisson", las = 1,
xlab="Location", ylab="Spikes on Basis of 2nd Pereiopod", main="Spikes on Basis of the 2nd
Pereiopod", sub="")

model2<-glm(spikes_on_basis_2nd_pereiopod~lenght_carapace_mm*location,
family="quasipoisson")

model2<-glm(spikes_on_basis_2nd_pereiopod~lenght_carapace_mm+location,
family="quasipoisson")
anova(model2, test="F")
summary(model2)
mc2 <- glht(model2, linfct=mcp(location="Tukey"))
summary(mc2)
plot(model2)
```

#Analysis of "spikes_on_ischium_2nd_pereiopod"

```
plot(spikes_on_ischium_2nd_pereiopod~location , family="quasipoisson", las = 1,
xlab="Location", ylab="Spikes on Ischium of 2nd Pereiopod", main="Spikes on Ischium of the
2nd Pereiopod", sub="")

model2<-glm(spikes_on_ischium_2nd_pereiopod~lenght_carapace_mm*location,
family="quasipoisson")

model2<-glm(spikes_on_ischium_2nd_pereiopod~lenght_carapace_mm+location,
family="quasipoisson")
anova(model2, test="F")
summary(model2)
mc2 <- glht(model2, linfct=mcp(location="Tukey"))
summary(mc2)
plot(model2)
```

#Analysis of "scaphocerite_ratio"

```
plot(scaphocerite_ratio~location, las = 1, xlab="Location", ylab="Scaphocerite Ratio",
main="Width of Scaphocerite", sub="")

model3<-lm(scaphocerite_ratio~lenght_carapace_mm*location)

model3<-lm(scaphocerite_ratio~lenght_carapace_mm+location)
anova(model3)
summary(model3)
mc3 <- glht(model3, linfct=mcp(location="Tukey"))
summary(mc3)
plot(model3)
```

#Analysis of "scapocerite_carapace_length_ratio"

```
plot(scapocerite_carapace_length_ratio~location , las = 1, xlab="Location", ylab="Ratio",
main="Scaphocerite and Carapace Length Ratio", sub="")

model4<-lm(scapocerite_carapace_length_ratio~lenght_carapace_mm*location)

model4<-lm(scapocerite_carapace_length_ratio~lenght_carapace_mm+location)
```

```
anova(model4)
summary(model4)
mc4 <- glht(model4, linfct=mcp(location='Tukey'))
summary(mc4)
plot(model4)
```

#Analysis of "lenght_ratio"
```
plot(lenght_ratio~location , las = 1, xlab="Location", ylab="Ratio", main="Total Length and
Carpa Length Ratio", sub="")

model5<-lm(lenght_ratio~lenght_carapace_mm*location)

model5<-lm(lenght_ratio~lenght_carapace_mm+location)
anova(model5)
summary(model5)
mc5 <- glht(model5, linfct=mcp(location='Tukey'))
summary(mc5)
plot(model5)
```

#Analysis of size distributions within groups
```
plot(lenght_carapace_mm~location , las = 1, xlab="Location", ylab="Carapace Length",
main="Size Distribution within Populations", sub="")
model5<-lm(lenght_carapace_mm~location)
anova(model5)
summary(model5)
mc5 <- glht(model5, linfct=mcp(location='Tukey'))
summary(mc5)
plot(model5)
```

5.2 Analyses of age influenced morphological change

#Analysis of "spikes_on_basis_2nd_pereiopod" and size
```
plot(lenght_carapace_mm, spikes_on_basis_2nd_pereiopod, las = 1, xlab="Length Carapace
(mm)", ylab="Number of Spikes on Basis of 2nd Pereiopod", main="Spikes on Basis of 2nd
Pereiopod and Size Relationship", sub="")
cor1 <- lm(spikes_on_basis_2nd_pereiopod~lenght_carapace_mm)
abline(cor1)
cor(lenght_carapace_mm, spikes_on_basis_2nd_pereiopod, use="complete")
plot(cor1)
```

#Analysis of "scaphocerite_ratio" and size
```
plot(lenght_carapace_mm, scaphocerite_ratio, las = 1, xlab="Length Carapace (mm)",
ylab="Scaphocerite Ratio", main="Scaphocerite Ratio and Size Relationship", sub="")
cor2 <- lm(scaphocerite_ratio~lenght_carapace_mm)
abline(cor2)
cor(lenght_carapace_mm, scaphocerite_ratio, use="complete")
plot(cor2)
```

#Analysis of "spikes_on_ischium_2nd_pereiopod" and size
```
plot(lenght_carapace_mm, spikes_on_ischium_2nd_pereiopod, las = 1, xlab="Length
Carapace (mm)", ylab="Spikes on Ischium of 2nd Pereiopod", main="Spikes on Ischium of
2nd Pereiopod and Size Relationship", sub="")
cor2 <- lm(spikes_on_ischium_2nd_pereiopod~lenght_carapace_mm)
abline(cor2)
cor(lenght_carapace_mm, spikes_on_ischium_2nd_pereiopod, use="complete")
plot(cor2)
```

#Analysis of rostrum type and size

```
pasiphaea.df$rostrum.bin <- ifelse(rostrum=="spiky_curved_up", 0,
ifelse(rostrum=="curved_up", 1/3, ifelse(rostrum=="straight", 2/3,
ifelse(rostrum=="curved_down",1, NA))))
attach(pasiphaea.df)
fit.glm <- glm(rostrum.bin~lenght_carapace_mm, family="quasibinomial")
anova(fit.glm, test="F")
plot(rostrum.bin~lenght_carapace_mm, xlab="Length carapace (mm)", ylab="Probability of
rostrum phenotype", main="Probability Distribution of Rostrum Phenotype given Size",
axes=F)
xvals <- seq(min(lenght_carapace_mm, na.rm=T), max(lenght_carapace_mm, na.rm=T),
0.01)
lines(xvals, predict(fit.glm, newdata=data.frame(lenght_carapace_mm=xvals),
type="response"))
axis(1)
axis(2, at=c(0,1/3, 2/3, 1), labels=c("Sp+CU", "CU", "ST", "CD"))
box()
```