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Barcode-based species delimitation in the marine realm: a test using Hexanauplia (Multicrustacea: Thecostraca and Copepoda)

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Abstract

DNA barcoding has been used successfully for identifying specimens belonging to marine planktonic groups. However, the ability to delineate species within taxonomically diverse and widely-distributed marine groups, such as the Copepoda and Thecostraca, remains largely untested. We investigate whether a cytochrome *c* oxidase subunit I (COI-5P) global pairwise sequence divergence threshold exists between intraspecific and interspecific divergences in the copepods plus the thecostracans (barnacles and allies). Using publicly accessible sequence data, we applied a graphical method to determine an optimal threshold value. With these thresholds, and using a newly-generated planktonic marine data set, we quantify the degree of concordance using a bi-directional analysis and discuss different analytical methods for sequence-based species delimitation (e.g. BIN, ABGD, jMOTU, UPARSE, Mothur, PTP, GMYC). Our results support a COI-5P threshold between 2.1 and 2.6% p-distance across methods for these crustacean taxa, yielding molecular groupings largely concordant with traditional, morphologically-defined species. The adoption of internal methods for clustering verification enables rapid biodiversity studies and the exploration of unknown faunas using DNA barcoding. The approaches taken here for concordance assessment also provide a more quantitative comparison of clustering results (as contrasted with "success/failure" of barcoding), and we recommend their further consideration for barcoding studies.

Keywords: Maxillopoda, DNA barcoding, MOTU, species delineation, species delimitation, coalescence

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Introduction

Quantifying biodiversity in marine ecosystems has become increasingly important due to a rise in anthropogenic disturbances and increases in local and global extinction rates (Singh 2002; Radulovici et al. 2010). Traditional morphological approaches to specimen identification are not realistic for completing comprehensive surveys of marine regions due to cost, expertise, and time required. Additionally, even when sufficient resources are available, morphological identifications are challenging for many specimens due to variation in morphological characters across life stages, poorly-resolved taxonomy in some groups, and cryptic or undescribed species (Knowlton 1993; McManus and Katz 2009; Packer et al. 2009). These difficulties are further exacerbated when conducting geographically-broad biodiversity surveys, as taxonomic expertise is often linked to specific taxonomic groups and/or geographic regions. As such, DNA sequence-based specimen identification systems, including DNA barcoding (Hebert et al. 2003a,b), are indispensable for conducting large-scale biodiversity surveys. Once established, molecular methodological pipelines, publicly accessible sequence databases, and tested analytical tools will not only facilitate biodiversity surveys but will also enable the rapid detection of introduced species through environmental sampling and high-throughput sequencing techniques (Cristescu 2014).

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Identifying unknown specimens through DNA barcodes requires a reference library containing morphologically-identified, barcoded specimens against which unknowns can be compared (Collins and Cruickshank 2014). Currently, it is challenging to use DNA barcode databases for identification of many marine species; most species remain to be described, and even known species often have little to no DNA barcode coverage (McManus and Katz 2009;

Bucklin et al. 2010; Blanco-Bercial et al. 2014). Important first steps for enabling specimen identification using DNA barcodes are to investigate patterns of interspecific and intraspecific variation within target taxa and to determine the degree to which an integrative approach to species delimitation is necessary—as contrasted with more straightforward approaches using a single molecular marker (Collins and Cruickshank 2013, 2014). In addition to building an identification system for known species, it is important to be able to determine when a specimen is likely to represent a species that is novel to the database through some form of species delimitation.

The success of DNA barcoding has been documented across a range of marine taxa (e.g. da Silva et al. 2011; Blanco-Bercial et al. 2014; and references therein). Despite these successes, establishing a robust system is still challenging. With 31 phyla—and with an estimated million species remaining to be discovered—the genetic variation within marine metazoans is not fully appreciated (Bucklin et al. 2010). Earlier molecular approaches to specimen identification relied on the presence of low intraspecific genetic variation and larger interspecific divergence between species, i.e. the presence of a "barcoding gap" (discussed in Meyer and Paulay 2005). Despite criticisms of strictly similarity-based approaches (Will et al. 2005; Hickerson et al. 2006; Collins and Cruickshank 2013), such delimitation methods have been shown to be useful among a wide range of taxa (Will et al. 2005; Ebach and Holdrege 2005; Radulovici et al. 2011; Huemer et al. 2014). Unfortunately, thresholds for species delimitation are often inappropriately chosen and simply based on past literature (Collins and Cruickshank 2013). Although there has been work developing methodologies to justify the selection of a molecular threshold (Lefébure et al. 2006; Blanco-Bercial et al. 2014), these methods rely solely on external comparisons, often only to taxonomic species identifications, which can vary widely depending on the identifier (Shen et al.

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2013). Nevertheless, once a well-defined and justified threshold is established, simple delimitation methods are advantageous for quickly and easily assessing potential biodiversity.

Here, our work focuses on the marine planktonic Copepoda and Thecostraca. Past placement of these classes was under the superclass Maxillopoda (Newman 1992). However, more recent work has indicated that the Maxillopoda is not monophyletic (Regier et al. 2005), and the taxonomic placement of Copepoda and Thecostraca within the arthropods has been redefined (Newman 1992; Regier et al. 2010; Oakley et al. 2013) (Fig. 1). Recent work using multiple lines of evidence has placed the subclasses Copepoda and Thecostraca as sister lineages making up Hexanauplia (Regier et al. 2010; Oakley et al. 2013).

We investigate the prospects for using rapid species delimitation tools within a hyper-abundant and widely-distributed group of marine invertebrates, the subclasses Copepoda and Thecostraca. Using publicly available COI-5P barcode data, we first describe patterns of genetic divergence and explore the potential for a global pairwise sequence divergence threshold to be used to delimit specimens into species-like units. Second, using a *novel* data set of morphologically-identified specimens, we also quantify the concordance among multiple sequence clustering methods as well as the concordance between molecular delimitations and current taxonomy. Our use of bidirectional concordances is a new approach for the barcoding literature. Our efforts provide insights into marine planktonic crustacean genetic divergence patterns and species boundaries under differing species definitions. In addition to contributing to the development of molecular identification systems for these taxa, the approaches employed here may be considered for other understudied marine invertebrates with large geographic ranges.

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Methods

Specimen collection and molecular laboratory methodology

Plankton samples were collected from May 2011 to August 2012 at one proposed port location and eleven current port locations across all three of Canada's ocean regions (Arctic, Atlantic, and Pacific) (Fig. 2). Plankton samples were collected from small vessels using plankton nets of both 250 µm and 80 µm mesh sizes at 0 - 15 m depth. At most locations, two seasonal periods were sampled: July - September and November - December. Collections in northern regions were sometimes limited to one season due to logistical challenges. At each location, for each season collected, six separate plankton tows were made to provide representation across the entire port.

Samples were maintained in 95% ethanol and transferred to -20 °C cold storage within six months of collection. All samples were split into three fractions, one of which was used in this study. To reduce the number of samples for sorting and identification, all samples from a single mesh size from a single port were combined, and for the remainder of this manuscript we refer to these as "samples". Specimens within samples were sorted morphologically and were taxonomically identified to the lowest possible level (identification references: Nouvel 1950; Gardner et al. 1982; Roff et al. 1984; Kathman 1986; Todd et al. 1996; Gerber 2000; Johnson 1996; Johnson et al. 2012). Four to six individuals of each morphologically-identified maxillopod taxon per sample were used for further molecular analysis.

DNA extraction consumed single whole individuals, as all individuals were less than approximately 1 mm^3. Batch vouchers were designated that consisted of individuals from the same site and assigned the same morphological identification as the sequenced specimen. The specimens will be archived at the Biodiversity Institute of Ontario, University of Guelph, and the digital specimen information is available through the Barcode of Life Data Systems (BOLD,

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http://www.boldsystems.org/, project code CAISN) (Ratnasingham and Hebert 2007). DNA extraction followed Ivanova et al. (2006) with several variations (specific methodology can be located in the supplementary file S6). Multiple PCR primer sets were used to amplify the animal barcode region. These primers and further details of the molecular methodology can also be found in the supplementary material (S6).

CodonCode Aligner (CodonCode Corporation) was used to display sequence quality, screen for short sequence amplifications, and assemble consensus sequences where available. Sequences were aligned using the default (FFT-NS-2) alignment strategy of the Multiple alignment program for nucleotide sequences (MAFFT Ver. 7) (Katoh and Standley 2013), and the multiple sequence alignment (MSA) was trimmed to a final length of 588 base pairs (bp). The MSA was then translated using the invertebrate mitochondrial code in MEGA6 (Tamura et al. 2013) for verification of the alignment. Single nucleotide insertions or deletions evident through frame shifts were further investigated and edited if they revealed an error in the nucleotide base reading. In cases of an unresolved frame shift or stop codon, the sequence was removed as this pattern suggests the presence of a nuclear pseudogene. All remaining sequences were screened for potential contamination and/or misidentifications, and specific methodology can be found in the supplementary material (S6).

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Data sets and molecular operational taxonomic units

Two data sets of COI-5P sequences were analyzed. The *novel* data set consisted of marine collections sequenced here that were morphologically identified to the species level. The second set, *reference*, contained all Copepoda and Thecostraca specimens collected here which were not able to be identified to the species level together with all publicly accessible

Maxillopoda data on the BOLD system (BOLD search for "Maxillopoda" in the public data portal, using the API search method conducted on December 1, 2014 http://www.boldsystems.org/index.php/API_Public/specimen?taxon=Maxillopoda). Although the taxonomic designation Maxillopoda is no longer accepted, it was necessary to use it when searching for public sequence data, as many databases have not been updated to the currently-accepted taxonomy. The two data sets were used in several different and several similar analyses, which are described below and visually displayed in Figure 3. The *reference* data set was then reduced by excluding those sequences not assigned a Barcode Index Number (BIN), to remove sequence data not meeting the minimum quality standards for BIN compliance (Ratnasingham and Hebert 2013). Genetic distances were calculated and summarized using the 'Distance Summary' and 'Barcode Gap Analysis' tools on BOLD (Ratnasingham and Hebert 2007). All sequences longer than 200 bp were analyzed for the *reference* and *novel* data sets. Analyses were conducted using the BOLD sequence alignment, K2P (Kimura 1980) genetic distances, and pairwise deletion of missing data.

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Five similarity-based and two coalescence-based analyses for generating molecular clusters or Molecular Operational Taxonomic Units (MOTUs) (Blaxter 2004) were compared. Similarity-based methods included: Barcode Index Number (BIN) (Ratnasingham and Hebert 2013), Automatic Barcode Gap Discovery (ABGD) (Puillandre et al. 2012), jMOTU (Jones et al. 2011), UPARSE (Edgar 2013), and Mothur (Schloss 2009). The two coalescence-based methods were Poisson Tree Processes (PTP) (Zhang et al. 2013) and Generalized Mixed Yule Coalescent (GMYC) (Fujisawa and Barraclough 2013). Coalescent-based phylogenetic methods were included in the comparison for the *novel* data set only, as the *reference* data set was too large and construction of an input tree too computationally expensive, while similarity-based methods were applied to both data sets. Prior to model testing and tree construction, all exact duplicate sequences were removed from the MSA using ElimDupes (https://hcv.lanl.gov/content/sequence/ELIMDUPES/elimdupes.html). Phylogenetic reconstruction for the *novel* data set used the best-fit model of nucleotide substitution as determined using the Bayesian Information Criterion (BIC) implemented in the jModelTest2 program (Guindon and Gascuel 2003; Darriba et al. 2012). Talavera et al. (2013) recently investigated the results from GMYC when using input trees variously constructed and largely found the same resulting clustering assignments. We also used three different tree-building methods (ultrametricized trees constructed using Bayesian, neighbour-joining, and maximum likelihood methods) for use by the coalescent-based MOTU delineation programs. As the clusters from GMYC, as well as PTP, across all three input trees were similar, detailed methods and results are presented for the Bayesian tree only (see supplementary file S6 for Bayesian tree construction details), consistent with recommendations by Tang et al. (2014). PTP and GMYC clustering results using unique haplotypes were used to assign all sequences/specimens in the data set to a cluster.

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The GMYC (Fujisawa and Barraclough 2013) analysis was conducted using python implementation of the single-threshold model as downloaded from the Exelixis Lab webserver (http://species.h-its.org/gmyc/). The Poisson Tree Processes (PTP) model was also used to infer putative species boundaries (Zhang et al. 2013). The BOLD-implemented refined single linkage (RESL) algorithm provided BIN assignments for each sequence (Ratnasingham and Hebert 2013). This method uses a 2.2% p-distance seed threshold, but then refines groupings for individual BINs and neighbouring clusters based on the level of continuity in the distribution of genetic divergences among sequences. The jMOTU program uses a similarity-based approach; a

BLAST identity filter of 99 was employed, and clusters were arranged using the number of variable nucleotides, equivalent to p-distance (Jones et al. 2011). For the remaining similarity-based methods (ABGD, Mothur, and UPARSE), we set the programs to define molecular clusters using p-distance so that results could be compared across methods (Puillandre et al. 2012; Schloss 2009; Edgar 2013).

The ABGD method was implemented through the ABGD C source available on the ABGD website (Puillandre et al. 2012). Analysis settings were: Pmin value of 0.001, Pmax equal to 0.15, a minimum gap width X equal to 0.001, with 1000 steps using p-distance. The gap width used here was much smaller than the default width of 1.5, and the number of steps was much larger at 1000 compared to 10. This was done to obtain clustering results across a range of divergences to conduct an elbow analysis (explained below). UPARSE implementation with threshold values greater than 3% is not recommended, and a workaround for this problem was implemented in order to explore a broad range of possible thresholds, which can be seen in the supplementary files (S1). The Mothur program had three clustering options available for MOTU assignment (nearest neighbour, furthest neighbour, and average neighbour), and the average neighbour method was used. Commands and scripts for the generation of the results from Mothur can be found in the supplementary files (S1).

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Determining optimal global thresholds

To calculate the optimal divergence threshold for each similarity-based clustering method, clusters were generated for the *reference* data set using a range between 0% and 15% p-distance pairwise divergence thresholds. This range was chosen as it is expected to encompass the general transition between intraspecific and interspecific variation within our COI-5P data set (Blanco-

Bercial et al. 2014). For each program for which the user can specify the threshold (ABGD, jMOTU, Mothur, and UPARSE), the numbers of clusters generated at each p-distance were plotted, and the vertex point of the resulting curve was considered to represent the optimal threshold for clustering these data for that method (Handl et al. 2005) (Fig. 4). For pairwise divergence thresholds below the proposed optimum, the sequences will be over-split into too many MOTUs, having lower correspondence to evolutionary species units. Conversely, at pairwise divergence thresholds above the optimum, sequences will be over-lumped into too few MOTUs. To determine this point, a graphical approach was employed where the Euclidian distance between the origin of the graph (0,0) and every point on the curve was obtained. The point on the curve with the smallest Euclidean distance to the origin was considered the hypothesized, ideal global threshold value (Fig. 4). To determine this threshold using empirical data, thereby foregoing the need to approximate the curve, the y-axis (number of MOTUs at a given threshold) was scaled to be equal in length to the x-axis. Analysis conducted using divergence values between 0-10% and 0-20% and scaling the y-axis similarly yielded very similar results (not shown).

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Concordance among MOTUs and between MOTUs and morphological species

Concordance among MOTUs generated by different analytical methods, as well as concordance between MOTUs and morphological species assignments, was quantified using an Adjusted Wallace coefficient (Wallace 1983). This coefficient was selected because it provides bi-directional results. Calculation of the coefficient required a data matrix containing all specimens, with each row representing a unique specimen and each column a unique clustering analysis result (see supplemental file, S1). Specimens were removed from the analysis if data were missing in any one of the clustering columns being analyzed. Once a matrix was constructed, Adjusted Wallace coefficients were computed through the website Comparing Partitions (http://darwin.phyloviz.net/ComparingPartitions/index.php?link=Home) (Severiano 2011). Coefficients were determined for the *reference* and *novel* data sets separately, and MOTUs were generated for each data set using the analysis-specific thresholds obtained from the larger (*reference*) data set only.

Comparing MOTUs and morphological identifications

Overall concordance between two clustering methods quantifies how well they agree; however, it does not provide information on the nature of the agreements/disagreements nor highlight those possible problematic sequences or taxa that are yielding the conflicting clustering results. To understand how clustering results obtained from the various molecular methods agreed with morphological groupings, we quantified molecular cluster agreement to all Linnaean species labels for both the *novel* and *reference* data sets. The comparison resulted in four possible outcomes: complete ‘match’ where both clusters match exactly; an exact ‘split’ where the reference cluster was split into multiple clusters, with no members of the corresponding clusters being unaccounted; a complete ‘lump’ where the reference cluster was combined with one or more additional reference clusters in their entirety, with no members unaccounted; and a ‘mixed’ result where a reference cluster was both split and lumped (Ratnasingham and Hebert 2013). Species represented by a single specimen were removed from this analysis, as these specimens are only able to represent matches in the analysis and would thus bias the results towards concluding concordance. An agreement matrix was constructed, and total match, split, lump, and mixed numbers were tabulated for each data set and clustering analysis.

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Results

Novel molecular data set of Canadian marine Hexanauplia

A total of 404 new DNA barcode sequences was generated. After applying a 500 bp length criterion and removing sequences containing more than 1% Ns, our study yielded 366 (247 identified to species) novel barcode sequences for a newly-sampled and morphologically-identified planktonic crustacean collection (see taxonomic breakdown table in supplementary file S1). Amplification of the COI-5P region was challenging, with some specimens receiving 8 PCR attempts using up to 6 different primer sets. Once a protocol was established, successful amplifications across the Hexanauplia data set increased, and final sequencing success was 59% of individuals, representing 100% of the morphological species. There were several groups which remained more difficult to amplify, including: *Calanus*, *Microcalanus, Metridia* (specifically *Metridia longa*), *Oithona*, *Paracalanus*, and *Pseudocalanus*.

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Patterns of genetic divergence

There was an overall separation between intraspecific vs. interspecific divergences for both the *reference* and *novel* data sets (Fig. 5). For the *reference* data set, the results were limited to sequences with associated Linnaean species names, which resulted in a data set of 2825 sequences comprised of 262 species. The average for the mean intraspecific K2P divergence values for all species in the *reference* data set was 1.84%. The mean of the maximum intraspecific pairwise distances was 3.03%, and the mean distance to the nearest neighbour divergence (the smallest pairwise distance to the closest individual of a different species) was 14.79%. The *novel* data set, with 247 sequences representing 27 species, had a mean maximum intraspecific distance of 4.35% and a mean distance to the nearest neighbour of 19.82%. The average for the mean intraspecific divergence values for all species in the *novel* data set was 1.81%.

Proposed threshold values through elbow analysis

Global pairwise sequence divergence (GPSD) thresholds, proposed to represent an optimum generally separating interspecific and intraspecific divergences, ranged from 2.1-2.6% across clustering analysis methods for the *reference* data set. Analysis with ABGD displayed the lowest percent divergences with a 2.1% result. The jMOTU and UPARSE analyses had threshold values of 2.3% and 2.2%, respectively, while the Mothur result had the highest value at 2.6%.

Concordance among MOTU clustering methods

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Molecular clustering results with 2825 specimens, representing 262 uniquely identified species, from the *reference* data set were similar across the four similarity-based methods using GPSD threshold values (Table 1). This concordance index can take values between 0 and 1, with higher values indicating a strong ability of one clustering approach (row label) to explain the clusters generated by another method (column label). Concordance values between a pair of methods can differ in accordance with the direction of the comparison.

The adjusted Wallace concordance values for the *novel* data set showed varied concordance across the eight clustering methods (Table 2). Due to the UPARSE function of identifying suspected chimeric sequences, three sequences were removed from all concordance calculations, leaving 244 specimens for analysis. There were noticeably directional results in discriminatory power between molecular clustering results and morphological Linnaean species labels. For example, the Wallace coefficient value from BINs to Linnaean species labels was 0.925, meaning that two specimens in the same BIN have a 92.5% chance they would also have the same Linnaean species label. By contrast, two specimens with the same Linnaean species label only have 56.2% chance of falling in the same BIN. This example is similar in all comparisons between morphological Linnaean species and molecular clusters, where the molecular clusters are more discriminant than the species designations. The PTP coalescence-based clustering of the specimens partitioned the data far more than all other methods, with 51 MOTUs, compared with 37-40 MOTUs for most of the molecular methods and 29 morphological species groups. Moreover, PTP had the lowest concordance in comparison to all other clustering results, including both molecular and morphological techniques. The morphological species were best able to explain the ABGD results, with a concordance value of 0.704, but generally exhibited more modest ability to explain the molecular results as compared to all other molecular clustering methods (0.664 - 0.819 for ABGD to explain other molecular clustering results, 0.994 - 1 for ABGD clusters to be explained by all other molecular clustering methods). Sets of clusters generated by BIN, jMOTU, Mothur, and UPARSE had the highest level of concordance with one another among all comparisons (0.988 - 1.00). Overall, molecular clustering methods, including both similarity-based and coalescence-based, had a strong ability to explain the morphological species; by contrast, the morphological species had a generally low ability to explain the molecular clustering results. This suggests molecular data are more discriminant, and generate more clusters, than the morphological data, as examined for assigning specimens to Linnaean species, for the *novel* data set.

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Results comparing molecular clusters to morphological groupings

The extent of agreement between molecular clusters and morphological species, containing more than one identified specimen, varied between the *reference* and *novel* data sets.

For the *novel* data set, the Linnaean names were assigned during this study and are based upon morphology; it is presumed this is primarily the case for the *reference* data set as well, but varied methods (including integrative consideration of molecular data) could have been used among the records in the public data set. The molecular-based clusters displayed between 40-80% direct matches with species labels in the *novel* data set and 55-66% using the *reference* data set (Fig. 6). Of the 29 morphologically-identified species in the *novel* data set, 20 were represented by more than one specimen and so were included in the agreement analysis. Ten morphologically-identified species shared a high degree of agreement between barcode-based clusters and morphological identifications. Two genera, *Acartia* and *Centropages*, showed varying degrees of agreement among clustering methods including mixed, split, or matched specimen clustering assignment. The species *Eurytemora herdmani* and *Tortanus discaudatus* were predominately split into two or more molecular clusters. The remaining identified species (*Paracalanus parvus, Temora longicornis, Zaus abbreviatus*) predominately matched molecular clusters. The *reference* data set exhibited 56.0% (153/273) exact matches across all clustering methods. Of the remaining Linnaean names, approximately 2.2% were predominantly matched to most molecular clustering methods, 11% were split, 2.9% were lumps, and 25.6% were mixes.

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Discussion

Here, we empirically estimate a COI-5P threshold for Hexanauplia, which ranges between 2.1 to 2.6% p-distance among the analytical methods employed. We then used these thresholds to quantify the degree to which molecular clusters agree with species units according to current taxonomy. We discovered that, for approximately 60% of Linnaean species labels, molecular species delineation methods assigned specimens into the same groupings as morphological identifications. We suggest that concordance of independent data sets can provide greater support for species boundaries, with more confidence that the groupings arising from these different character sets reflect evolutionarily independent species (see Mayden 1997 for treatment of species concepts).

Support for rapid species delimitation for Hexanauplia using global thresholds

Although the use of GPSD thresholds has been criticised (Will et al. 2005; Ebach and Holdrege 2005), taxon-specific studies have established that thresholds may successfully delimit specimens into clusters that are largely concordant to established taxonomic groups (Lefébure 2006; Ratnasingham and Hebert 2013; Huemer et al. 2014). Unfortunately, research to test the performance of COI barcode data in marine invertebrate taxa has been limited (but see Radulovici et al. 2010; Bucklin et al. 2010, 2011; Blanco-Bercial et al. 2014). Furthermore, studies investigating barcode performance often do so by comparing the relative performance of barcode data against morphologically-identified specimens. The primary use of morphological identification as the gold standard for concluding "successes" and "failures" of barcode data, which is common but not universal across the barcoding literature, presents problems with marine taxa such as Hexanauplia, as some reproductively, evolutionarily, and even ecologically distinct taxa lack discernible and diagnostic morphological differences (Carrillo et al. 1974; Knowlton 1993; McManus and Katz 2009; Bucklin et al. 2011). Few studies have tested the utility of barcode data for providing consistent clustering through an internal, sequence-based measure of cluster validation (Handl et al. 2005).

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Establishing a threshold for hexanauplian COI-5P barcode clusters, done here through elbow analysis, can provide an appropriate single threshold for the taxon as a whole. By basing this analysis upon similarity-based clustering methods, a threshold can be calculated much more efficiently than by applying coalescence-based methods, which are not presently feasible for extremely large data sets. This graphical method yields clusters similar to Linnaean taxonomy, but more finely sub-divided. This is evident in the high unidirectional Adjusted Wallace coefficient values, whereas Linnaean values are lower compared to molecular values. This increased resolution by molecular clustering still allows for MOTUs to be linked back to current taxonomy, which is especially important for conservation studies and when screening for species presence/absence as part of invasive species monitoring efforts. Our molecular clustering approaches are partitioning the data into clusters smaller than current morphological species descriptions; for some study aims, a less stringent GPSD threshold may be needed if delimitation into current morphological species groups is desired as opposed to expected diversity of evolutionarily distinct species based on molecular data.

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The similarity-based molecular methods yielded similar results to GMYC, in which a threshold is sought which explicitly divides nodes into those corresponding with Yule (inter-species) vs. coalescent (intra-species) evolutionary processes. Therefore, both of these categories of methods may yield groupings that are closer to evolutionary species, a species concept which is increasingly favoured by several authors (e.g. Mayden 1997; de Queiroz 2007), than they are to morphological species. While barcode-based MOTUs may represent evolutionarily distinct but in some cases morphologically similar species, further study based on additional molecular, morphological, and biological data is generally considered necessary before formal taxonomic revisions are supported (Collins and Cruickshank 2013). Researchers may also disagree about the biological meaning of divergent allopatric mitochondrial lineages within single Linnaean species, depending upon the preferred species concept. While a demonstration of reproductive isolation in sympatry may be required to meet species status under the biological species concept, separately evolving, genetically divergent populations that are currently on different evolutionary trajectories might be recognized as species under an evolutionary species concept (Mayden 1997). Nevertheless, our results support the use of a single molecular marker for rapid species delineation and for indicating likely cryptic species for further exploration. We sampled up to 34 individuals for those Linnaean species which were split into two or more MOTU here. As these exhibited up to 10% sequence divergence, we suggest that further study is warranted as some of these may meet the criterion for species status under biological or evolutionary species concepts.

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We have presented results using limited specimen collections (see taxonomic breakdown table in supplementary file S1) of Hexanauplia, and there could exist differing levels of molecular variation for sub groups within this focal taxon. However, this was not tested here as we were interested in a possible GPSD threshold which would partition the data into species-like groups across a large taxonomic data set. Although there may be some taxonomic bias present in our data sets, there is a large phylogenetic breadth in the *reference* data set, particularly for Calanoida and Sessilia. Given the diverse data set, we expect that the global threshold will also work well across other poorly sampled groups in our data sets, such as Poecilostomatoida and Harpacticoida, although this supposition would benefit from directed testing in future studies. Having evidence to support a single threshold for larger taxonomic breadth is important, as this is desirable for analyses based upon high-throughput sequencing of mixed-species samples.

It is also important to note that there could be noise in our results potentially due to misidentified specimens. It is likely that some misidentifications are present in our data sets. This is especially true of the *reference* data set obtained from the publically accessible BOLD data base. It is unlikely that such misidentifications would greatly influence the outcome of our elbow analysis, as this analysis only relied upon class-level identifications. However, the presence of misidentifications can more significantly impact our concordance and agreement analyses. This issue may explain why we observed a higher proportion of “lumps” in the *reference* dataset compared to our *novel* dataset. Misidentifications present in the *novel* data set would again add error to our agreement analysis comparing molecular clustering results with morphology-based identifications. Although not completed here due to limited resources, it would be beneficial to verify the accuracy of our morphological identifications. This could be accomplished by using the remaining batch vouchers as the basis for multiple sets of independent identifications by different investigators, followed by DNA barcoding of these specimens.

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Current suggestions in the literature indicate the use of a Maxillopoda threshold ranging from 2-3% pairwise divergence (Radulovici et al. 2010; Blanco-Bercial et al. 2014). Here, the total range of determined thresholds for both data sets across similarity-based methods was fairly small, between 2.1 and 2.6% GPSD (p-distance) depending upon the method, and consistent with previous reports. This range, which was obtained using the same data set, shows how the choice of clustering analysis can impact the resulting clusters. Interestingly, the BIN 2.2% seed threshold—which was calibrated against morphological species using select groups of taxa: bees, butterflies and moths, fish, and birds (Ratnasingham and Hebert 2013)—is within the range of global thresholds calculated here. Results presented here show that GPSD thresholds are relatively consistent across the four tested similarity-based analyses and that the following GSPD thresholds should be used for copepods and thecostracans (ABGD = 2.05%; jMOTU= 2.35%; Mothur= 2.6%; UPARSE= 2.2%).

Similarity-based vs. coalescent-based methods: Performance and feasibility

Similarity-based delimitation methods have certain advantages over coalescence-based methods: speed, simplicity to implement, and ability to accommodate large data sets. Among the taxa investigated here, the similarity-based delimitation methods (ABGD, jMOTU, Mothur, UPARSE, and BINs) displayed a higher concordance compared to morphology than did coalescence-based methods (PTP and GMYC). Specifically, BINs exhibited more direct matches to Linnaean names than PTP and GMYC results. This slightly better performance using the BIN method than the other clustering methods, when compared to current taxonomy, has also been reported in fish, birds, and two moth groups (Ratnasingham and Hebert 2013).

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Ratnasingham and Hebert (2013) report an overall higher percentage of exact cluster matches between BINs and Linnaean species than we report (Ratnasingham and Hebert: 83-97%, here: 40-80%). This difference is most likely indicating a lower correspondence in Hexanauplia between named species and evolutionary species—those currently on separate evolutionary trajectories as indicated by genetic separation, whether allopatric or not. This could also be due to the presence of more readily discernible diagnostic morphological characters in those taxonomic groups or a higher proportion of species pairs in Hexanauplia that are only distinguishable by chemical, ecological, and/or behavioural traits (e.g. see Knowlton 1993).

There were two pairs of taxa exhibiting mixing in our *novel* data set *(Acartia hudsonica* with *A.longiremis*, *Centropages abdominalis* with *C. hamatus*). Although DNA barcodes have not been previously reported for *A. hudsonica* and *A. longiremis*, prior research using morphological and molecular evidence has shown close relationships among other *Acartia* species and high barcode variability within single *Acartia* species (Blanco-Bercial et al. 2014; da

Costa et al. 2011). As with *Acartia*, the genus *Centropages* has also been noted as having discordant molecular clustering as compared to morphological identifications (Blanco-Bercial et al. 2014). In addition, the Centropagidae family has been noted as having a plastic response to differing environments, thereby making morphological identifications more difficult (Blanco-Bercial et al. 2011). Although beyond the scope of this work, we suggest that future research is warranted into the specific nature of these 'mixing' results and the reasons for the discordance between molecular clusters and morphological species in these genera.

A lower proportion of direct matches between morphology and barcode-based clusters in copepods and thecostracans, compared to several better-studied taxa, may also be exacerbated by evolutionary processes that are largely unique to the marine realm, such as the accumulation of high levels of intraspecific diversity in extremely large populations with large geographic ranges. Due to this effect, combined with some cases of true recent speciation, Meyer and Paulay (2005) predicted greater overlap between intraspecific and interspecific divergences in marine environments as compared to terrestrial environments. Further investigation into this potential difference in patterns of sequence variation between marine and terrestrial systems is warranted and should include a broad variety of marine taxa. The optimal method for delimiting marine species may vary depending on the scientific questions and species concept. While in some instances the method of delimitation may be important, in others there may be little variation in the species counts across methods. This limited variation can be seen in our results where GMYC, BIN, jMOTU, Mothur, and UPARSE had very close concordance values, varying between approximately 0.88 and 1.0. This finding suggests that more rapid similarity-based methods are expected to yield groupings that largely correspond to the GMYC method, which is often favoured for its explicit evolutionary model. Consideration for the study question is also

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important; for example, a more discriminant analysis option or threshold may be preferable for detecting invasive species or endangered species in a given habitat.

Quantifying concordance as an alternative to concluding failure/success of barcoding

Assessing "true" species boundaries—especially in a geographically widespread and taxonomically diverse group such as Hexanauplia—is difficult, and boundaries can differ according to the preferred primary species concept and the selected character system (Mayden 1997; De Queiroz 2007). By quantifying concordance, we can examine and compare the signal for various delineations emerging from different character types and analysis methods. This bi-directional concordance assessment, not previously used in the barcoding literature, provides more information than simply reporting failures when molecular clusters do not agree with morphological species. This extra information can support existing morphological species boundaries as evolutionary species, through a new character system, or provide new biological insights (e.g. into potential cryptic species prevalence) in cases of discordance.

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Adjusted Wallace concordance values indicated that the molecular clusterings for the *novel* data set were more discriminant than current Linnaean species across all clustering analyses. This is not surprising as the low overall knowledge of the total biodiversity in Copepoda has been noted in the literature (Bucklin et al. 2011; Blanco-Bercial et al. 2014). Moreover, although morphological identifications for the *novel* data set were conducted by trained experts, some of the observed cases of discordance may be due to incorrect identification of specimens, due to the difficult taxonomy because specimens are often damaged during collection or preservation and juveniles have undeveloped diagnostic morphological characters. Using the *novel* data set, there was variation in clustering outcomes between coalescence-based and similarity-based analyses. Linnaean species better explain the similarity-based molecular clusters but are less concordant with the coalescent-based clusters. These results reflect some variation in the MOTUs generated using coalescence versus similarity-based analyses, with the latter generating groupings that can be somewhat better predicted by current species identifications. However, with the exception of PTP and ABGD, the differences among all molecular methods in their concordance to current taxonomy were modest. Thus, the molecular data appear to be revealing biological variation that was previously unrecognized in the current taxonomic hierarchy, which may be reflective of evolutionary species diversity.

Consistency among molecular delimitation methods

Concordance values provide an overall description of the consistency of the clustering results among methods; however, this metric tells us little about the specifics of how individuals are partitioned into clusters. If we first consider the *novel* data, when the clustering method results in numerous MOTUs there are also fewer exact matches between molecular groupings and Linnaean species. Although the variation in the number of clusters did appear to influence the proportion of matches to current species, the number of matches for all analyses was relatively similar, with between 60-70% exact matches. ABGD, with the fewest total clusters, had the highest number of exact matches among all other methods for the *novel* data set, with no splits. It is important to note that with a larger gap parameter setting than used here (such as the default) and recursive partitioning, the ABGD program performs an exploration of the variation in the data to create molecular groupings. As such, we found default parameters had a tendency to lump the data in to larger groups (results not shown). This may have been because our marine focal taxon exhibits more continuity in the range of genetic divergences than many taxa

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previously tested. Instead, we used a small gap parameter to force a threshold upon ABGD and obtain a specific global threshold using the elbow analysis approach. Therefore, ABGD may behave differently from here in future applications, depending upon the settings selected.

The remaining analyses (BIN, GMYC, jMOTU, Mothur, UPARSE) had very consistent agreement when considering both total matches and splits together (Fig. 6). These consistent results indicate the clustering methods, whether resulting in a match or split, can accurately place specimens into Linnaean species using these methods in approximately 70-80% of cases. If we were to remove taxa showing highly unstable correspondence between molecular groupings and current species, which are likely in need of taxonomic revision (*Acartia hudsonica, A. longiremis, Centropages abdominalis, C. hamatus, Eurytemora herdmani, Temora longicornis*), then the number of exact matches to current species for our methods would increase to approximately 78 and 91%, similar to barcode agreements reported in other taxonomic groups (Ratnasingham and Hebert 2013; Blanco-Bercial et al. 2014).

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Conclusion

We conclude that when applying GPSD thresholds that the method selected for MOTU generation is important; our results indicate a need for careful selection of both the method of generating MOTU clusters and the threshold applied. Our study has also found a larger number of MOTUs generated as compared to morphological species labels. These data indicate either poor taxonomic identification in the databases, the presence of cryptic species, and/or evidence of substantial intraspecific diversity at the COI-5P gene region. Continued research is needed to quantify to what extent these MOTUs represent real biological entities under an explicit species concept of interest. Future work may also include the amplification of additional molecular markers (particularly from the nuclear genome) to verify the taxonomic placement of specimens and lend support toward the identified GPSD thresholds for the COI-5P molecular region. This work may be especially useful for specimens identified here as problematic taxa. In addition to further research on species boundaries in taxonomically problematic groups, additional protocol development is also needed for groups with low amplification and sequencing success. This may include primer design and investigation of the importance of specimen fixation in cold conditions immediately following field collection (Prosser et al. 2013). Finally, the adoption of internal methods for clustering verification, such as the analyses presented here, is encouraged in DNA barcode studies to enable rapid biodiversity study and exploration of unknown faunas.

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References

Appeltans, W., Bouchet, P., Boxshall, G.A., Fauchald, K., Gordon, D.P., Hoeksema, B.W., ... and Costello, M.J. 2012. World register of marine species. Accessed online: http://www.marinespecies.org [accessed on 16 May 2016].

Blanco-Bercial, L., Bradford-Grieve, J., and Bucklin, A. 2011. Molecular phylogeny of the Calanoida (Crustacea: Copepoda). *Mol. Phylogenet. Evol.*, **59**(1): 103-113. doi:10.1016/j.ympev.2011.01.008

Blanco-Bercial, L., Cornils, A., Copley, N., and Bucklin, A. 2014. DNA barcoding of marine copepods: assessment of analytical approaches to species identification. *PLoS Curr*, **6**: 1–21. doi:10.1371/currents.tol.cdf8b74881f87e3b01d56b43791626d2

Blaxter, M.L. 2004. The promise of a DNA taxonomy. *Philos. Trans. R. Soc., B*, **359**(1444): 669–679. doi:10.1098/rstb.2003.1447

Bucklin, A., Hopcroft, R.R., Kosobokova, K.N., Nigro, L.M., Ortman, B.D., Jennings, R.M., and Sweetman, C.J. 2010. DNA barcoding of Arctic Ocean holozooplankton for species identification and recognition. *Deep Sea Res., Part II*, **57**(1–2): 40–48. doi:10.1016/j.dsr2.2009.08.005

Bucklin, A., Steinke, D., and Blanco-Bercial, L. 2011. DNA Barcoding of Marine Metazoa. *Annu. Rev. Mar. Sci.,* **3**(1): 471–508. doi:10.1146/annurev-marine-120308-080950

Carrillo, B.G., Miller, C.B., and Wiebe, P.H. 1974. Failure of interbreeding between Atlantic and Pacific populations of the marine calanoid copepod *Acartia clausi* Giesbrecht. *Limnol. Oceangr.*, **19**(3): 452–458. doi:10.4319/lo.1974.19.3.0452

Collins, R.A., and Cruickshank, R.H. 2013. The seven deadly sins of DNA barcoding. *Mol. Ecol. Resour.*, **13**(6): 969–975. doi:10.1111/1755-0998.12046

Collins, R.A., and Cruickshank, R.H. 2014. Known knowns, known unknowns, unknown unknowns and unknown knowns in DNA barcoding: a comment on Dowton et al. *Syst. Biol.*, **63**(6): 1005–1009. doi:10.1093/sysbio/syu060

Costa, F.O., DeWaard, J.R., Boutillier, J., Ratnasingham, S., Dooh, R.T., Hajibabaei, M., and Hebert, P.D.N. 2007. Biological identifications through DNA barcodes: the case of the Crustacea. *Can. J. Fish. Aquat. Sci.*, **64**(2): 272–295. doi:10.1139/F07-008

Cristescu, M.E. 2014. From barcoding single individuals to metabarcoding biological communities: towards an integrative approach to the study of global biodiversity. *Trends Ecol. Evol.*, **29**(10): 566–571. doi:10.1016/j.tree.2014.08.001

Draft

da Costa, K.G., Vallinoto, M., and da Costa, R.M. 2011. Molecular identification of a new cryptic species of *Acartia tonsa* (Copepoda, Acartiidae) from the Northern coast of Brazil, based on mitochondrial COI gene sequences. *J. Coast. Res.*, **S1**(64): 359-363.

da Silva, J.M., Creer, S., Dos Santos, A., Costa, A.C., Cunha, M.R., Costa, F.O., and Carvalho, G.R. 2011. Systematic and evolutionary insights derived from mtDNA COI barcode diversity in the Decapoda (Crustacea: Malacostraca). *PLoS ONE*, **6**(5): e19449. doi:10.1371/journal.pone.0019449

Darriba, D., Taboada, G.L., Doallo, R., and Posada, D. 2012. jModelTest 2: more models, new heuristics and parallel computing. *Nat. Methods*, **9**(8): 772–772. doi:10.1038/nmeth.2109

De Queiroz, K. 2007. Species concepts and species delimitation. *Syst. Biol.*, **56**(6): 879–886. doi:10.1080/10635150701701083

Ebach, M.C., and Holdrege, C. 2005. DNA barcoding is no substitute for taxonomy. *Nature*, **434**(7034): 697–697. doi:10.1038/434697b

Edgar, R.C. 2013. UPARSE: highly accurate OTU sequences from microbial amplicon reads. *Nat. Methods*, **10**(10): 996–998. doi:10.1038/nmeth.2604

Fujisawa, T., and Barraclough, T.G. 2013. Delimiting Species Using Single–locus Data and the Generalized Mixed Yule Coalescent (GMYC) Approach: A Revised Method and Evaluation on Simulated Data sets. *Syst. Biol.*, **62**: 707–724. doi:10.1093/sysbio/syt033

Gardner, G.A., and Szabo, I. 1982. *British Columbia pelagic marine Copepoda: an identification manual and annotated bibliography* (Vol. 62). Department of Fisheries and Oceans. Canadian Special Publication of Fisheries and Aquatic Sciences 62. Gerber, R.P. 2000. *An identification manual to the coastal and estuarine zooplankton of the Gulf of Maine region from Passamaquoddy Bay to Long Island Sound*. Parts 1 and 2. Acadia Productions, 80 and 98.

Guindon, S., and Gascuel, O. 2003. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Syst. Biol.*, **52**(5): 696–704. doi:10.1080/10635150390235520

Handl, J., Knowles, J., and Kell, D.B. 2005. Computational cluster validation in post-genomic data analysis. *Bioinformatics*, **21**(15): 3201–3212. doi:10.1093/bioinformatics/bti517

Hebert, P.D.N., Cywinska, A., Ball, S.L., and deWaard, J.R. 2003a. Biological identifications through DNA barcodes. *Proc. R. Soc. London, Ser. B*, **270**(1512): 313–321. doi:10.1098/rspb.2002.2218

Hebert, P.D.N., Ratnasingham, S., and deWaard, J.R. 2003b. Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species. *Proc. R. Soc. London, Ser. B*, **270**(Suppl 1): S96–S99. doi:10.1098/rsbl.2003.0025

Draft

Hickerson, M.J., Meyer, C.P., and Moritz, C. 2006. DNA barcoding will often fail to discover new animal species over broad parameter space. *Syst. Biol.*, **55**(5): 729–739. doi:10.1080/10635150600969898

Huemer, P., Mutanen, M., Sefc, K.M., and Hebert, P.D.N. 2014. Testing DNA barcode performance in 1000 species of European Lepidoptera: large geographic distances have small genetic impacts. *PloS ONE*, **9**(12): e115774. doi:10.1371/journal.pone.0115774

Ivanova, N.V., deWaard, J., and Hebert, P.D.N. 2006. An inexpensive, automation–friendly protocol for recovering high-quality DNA. *Mol. Ecol. Notes,* **6**(4): 998–1002. doi:10.1111/j.1471-8286.2006.01428.x

Johnson, W.S., and Allen, D.M. 2012. Zooplankton of the Atlantic and Gulf coasts: a guide to their identification and ecology. The Johns Hopkins University Press.

Johnson, K.B. 1996. A guide to marine coastal plankton and marine invertebrate larvae. Kendall Hunt.

Jones, M., Ghoorah, A., and Blaxter, M. 2011. jMOTU and Taxonerator: turning DNA Barcode sequences into annotated operational taxonomic units. *PloS ONE*, **6**(4): e19259. doi:10.1371/journal.pone.0019259

Kathman, R.D. 1986. Identification manual to the Mysidacea and Euphausiacea of the northeast Pacific (Vol. 93). Fisheries and Oceans, Information and Publications Branch.

Katoh, K., and Standley, D.M. 2013. MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. *Mol. Biol. Evol.*, **30**(4): 772–780. doi:10.1093/molbev/mst010

Kimura, M. 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J. Mol. Evol.*, **16**(2): 111–120.

Knowlton, N. 1993. Sibling species in the sea. *Annu. Rev. Ecol. Syst.*, **24**: 189–216.

Lefébure, T., Douady, C.J., Gouy, M., and Gibert, J. 2006. Relationship between morphological taxonomy and molecular divergence within Crustacea: proposal of a molecular threshold to help species delimitation. *Mol. Phylogenet. Evol.*, **40**(2): 435–447. doi:10.1016/j.ympev.2006.03.014

Mayden, R.L. 1997. A hierarchy of species concepts: the denouement in the saga of the species problem. In: Species: The Units of Biodiversity (Claridge, M.F., Dawah, H.A., and Wilson, M.R., Eds.), 381–324. Chapman and Hall, London.

McManus, G.B., and Katz, L.A. 2009. Molecular and morphological methods for identifying plankton: what makes a successful marriage? *J. Plankton Res.*, **31**(10): 1119–1129. doi:10.1093/plankt/fbp061

Draft

Meyer, C.P., and Paulay, G. 2005. DNA barcoding: error rates based on comprehensive sampling. *PLoS Biol.*, **3**(12): e422. doi:10.1371/journal.pbio.0030422

Nouvel, H. 1950. Mysidacea. Zooplankton No. 18–27. Conseil International Pour L'exploration De la Mer.

Packer, L., Gibbs, J., Sheffield, C., and Hanner, R. 2009. DNA barcoding and the mediocrity of morphology. *Mol. Ecol. Resour.*, **9**(s1): 42–50. doi:10.1111/j.1755-0998.2009.02631.x

Prosser, S., Martínez-Arce, A., and Elías-Gutiérrez, M. 2013. A new set of primers for COI amplification from freshwater microcrustaceans. *Mol. Ecol. Resour.*, **13**(6): 1151–1155. doi:10.1111/1755-0998.12132

Puillandre, N., Lambert, A., Brouillet, S., and Achaz, G. 2012. ABGD, Automatic Barcode Gap Discovery for primary species delimitation. *Mol. Ecol.*, **21**(8): 1864–1877. doi:10.1111/j.1365-294X.2011.05239.x

Radulovici, A.E., Archambault, P., and Dufresne, F. 2010. DNA Barcodes for Marine Biodiversity: Moving Fast Forward? *Diversity*, **2**(4): 450–472. doi:10.3390/d2040450

Rambaut, A., Suchard, M.A., Xie, D., and Drummond, A.J. 2014. Tracer v1.6, Available from http://beast.bio.ed.ac.uk/Tracer

Ratnasingham, S., and Hebert, P.D.N. 2007. BOLD: The Barcode of Life Data System. *Mol. Ecol. Notes*, **7**(3): 355–364. doi:10.1111/j.1471-8286.2007.01678.x

Ratnasingham, S., and Hebert, P.D.N. 2013. A DNA-based registry for all animal species: The Barcode Index Number (BIN) System. *PloS ONE*, **8**(7): e66213. doi:10.1371/journal.pone.0066213

Renaud, A.K., Savage, J., and Adamowicz, S.J. 2012. DNA barcoding of Northern Nearctic Muscidae (Diptera) reveals high correspondence between morphological and molecular species limits. *BMC Ecol.*, **12**(1): 24. doi:10.1186/1472-6785-12-24

Roff, J.C., Davidson, K.G., Pohle, G., and Dadswell, M.J. 1984. A guide to the marine flora and fauna of the Bay of Fundy and Scotian Shelf: larval Decapoda: Brachyura. Canadian Technical Report of Fisheries and Aquatic Sciences, 1322: iv + 57.

Saitou, N., and Nei, M. 1987. The Neighbor-joining Method: A New Method for Reconstructing Phylogenetic Trees. *Mol. Biol. Evol.*, **4**(4): 406–425.

Schloss, P.D., Westcott, S.L., Ryabin, T., Hall, J.R., Hartmann, M., Hollister, E.B., ... and Weber, C.F. 2009. Introducing Mothur: Open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Appl. Environ. Microbiol.*, **75**(23): 7537–7541. doi:10.1128/AEM.01541-09

Draft

Severiano, A., Pinto, F.R., Ramirez, M., and Carriço, J.A. 2011. Adjusted Wallace coefficient as a measure of congruence between typing methods. *J. Clin. Microbiol.*, **49**(11): 3997–4000. doi:10.1128/JCM.00624-11

Shen, Y.Y., Chen, X., and Murphy, R.W. 2013. Assessing DNA barcoding as a tool for species identification and data quality control. *PLoS ONE*, **8**(2): e57125. doi:10.1371/journal.pone.0057125

Shih, C.T. 1977. A guide to the jellyfish of Canadian Atlantic waters (No. 5). National Museum of Natural Sciences. Natural History Series, **5**: 90.

Sites, J.W., and Marshall, J.C. 2003. Delimiting species: a Renaissance issue in systematic biology. *Trends Ecol. Evol.*, **18**(9): 462–470. doi:10.1016/S0169-5347(03)00184-8

Srivathsan, A., and Meier, R. 2012. On the inappropriate use of Kimura-2-parameter (K2P) divergences in the DNA-barcoding literature. *Cladistics*, **28**(2): 190–194. doi:10.1111/j.1096-0031.2011.00370.x

Talavera, G., Dincă, V., and Vila, R. 2013. Factors affecting species delimitations with the GMYC model: insights from a butterfly survey. *Meth. Ecol. Evol.*, **4**(12): 1101–1110. doi:10.1111/2041-210X.12107

Tamura, K., Stecher, G., Peterson, D., Filipski, A., and Kumar, S. 2013. MEGA6: Molecular evolutionary genetics analysis version 6.0. *Mol. Biol. Evol.*, **30**(12): 2725–2729. doi:10.1093/molbev/mst197

Tang, C.Q., Humphreys, A.M., Fontaneto, D., and Barraclough, T.G. 2014. Effects of phylogenetic reconstruction method on the robustness of species delimitation using single-locus data. *Meth. Ecol. Evol.*, **5**(10): 1086–1094. doi:10.1111/2041-210X.12246

Todd, C.D., Laverack, M.S., and Boxshall, G. 1996. Coastal marine zooplankton: a practical manual for students. Cambridge University Press.

Wallace, D.L. 1983. A method for comparing two hierarchical clustering's: comment. *J. Am. Stat. Assoc.*, **78**(383): 569–576. doi:10.2307/2288118

Will, K.W., Mishler, B.D., and Wheeler, Q.D. 2005. The perils of DNA barcoding and the need for integrative taxonomy. *Syst. Biol.*, **54**(5): 844–51. doi:10.1080/10635150500354878

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

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TABLE 1. Adjusted Wallace coefficient (Wallace 1983) concordance values for the clustering results obtained from four programs for the *reference* data set. Thresholds applied to determine clusters are indicated in the row and column labels and were obtained for each analysis method via elbow analysis (Fig. 4). Values in parentheses indicate the total number of MOTUs as determined by the corresponding analysis and threshold. Each value in the table indicates how well the clusters generated by the method indicated by the row label correspond to the clusters yielded by the method indicated in the column label. Each pair of methods is represented by two values in the table.

	ABGD 2.1% (759)	jMOTU 2.3% (862)	Mothur 2.6% (856)	UPARSE 2.2% (878)
ABGD 2.1%	--	0.73	0.72	0.65
jMOTU 2.3%	1	--	0.99	0.89
Mothur 2.6%	1	1	--	0.89
UPARSE 2.2%	1	1	1	--

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TABLE 2. Bidirectional concordance among clustering methods for the *novel* data set using Adjusted Wallace's coefficients (Wallace 1983). Values in parentheses indicate the total number of clusters generated for each analysis. The global pairwise sequence divergence (GPSD) thresholds for ABGD, jMOTU, Mothur, and UPARSE are those obtained via elbow analysis using the *reference* data set. Each value in the table indicates how well the clusters generated by the method indicated by the row label correspond to the clusters yielded by the method indicated in the column label. Each pair of methods is represented by two values in the table.

	Morphology (29)	BINs (40)	PTP-ML (51)	GMYC (37)	ABGD 2.1 (30)	jMOTU 2.3 (40)	Mothur 2.6 (39)	UPARSE 2.2 (39)
Morphology (29)	--	0.562	0.46	0.52	0.704	0.563	0.569	0.559
BINs (40)	0.925	--	0.782	0.881	1	1	1	0.994
PTP-ML (51)	0.923	0.953	--	0.964	0.999	0.954	0.967	0.954
GMYC (37)	0.929	0.956	0.859	--	0.994	0.957	0.969	0.951
ABGD 2.1 (30)	0.938	0.81	0.664	0.742	--	0.81	0.819	0.806
jMOTU 2.3 (40)	0.926	0.999	0.783	0.881	1	--	1	0.994
Mothur 2.6 (39)	0.926	0.988	0.785	0.882	1	0.989	--	0.983
UPARSE 2.2 (39)	0.925	0.999	0.787	0.88	1	1	1	--

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FIGURE 1. A phylogram showing the relationship of Thecostraca and Copepoda with respect to other major arthropod lineages. Topology is based on Regier et al. (2010) and Oakley et al. (2013). The section of the tree shaded in blue indicates the clade Hexanauplia (Oakley et al. 2013) and represents the taxonomic focus of this work.

FIGURE 2. Map of Canada with plankton sampling sites indicated by red circles. Sites include: A - Vancouver, B - Victoria, C - Roberts Bank, D - Nanaimo, E - Churchill, F - Steensby Inlet, G - Iqaluit, H - Deception Bay, I - Baie de Sept-Iles, J - Port Hawksbury, K - Bedford Basin, L - Bayside.

FIGURE 3. Flow chart for the analysis of the two study data sets used in this study. Part 1 shows the key steps used to analyze the *reference* data set. Clustering analyses included the use of 4 programs: ABGD, jMOTU, Mothur, UPARSE. In addition to these four methods, BOLD BIN assignments were used to evaluate agreement to taxonomic identifications. Part 2 shows the steps in the analysis of the *novel* data set. The *novel* data set was clustered into MOTUs using the same four similarity-based analyses as for the *reference* data set (ABGD, jMOTU, Mothur, UPARSE). In addition to these, BINs and two coalescent (GMYC and PTP) clustering methods were used, and agreement to taxonomic identifications was quantified.

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FIGURE 4. Conceptual diagram for the determination of the optimal molecular divergence threshold values. The vertical long-dashed black line indicates the point on the curve (elbow) representing a threshold value that does not over-split or over-lump the sequences into MOTUs. This point represents the closest distance to the origin (0,0) (red circular-dashed arrow), as contrasted with larger vectors (blue small-dotted arrows). The corresponding point on the x-axis indicates the value for the percent pairwise divergence representing the proposed optimal threshold for given data set using the graphed analysis method.

FIGURE 5. Histograms displaying the intraspecific and interspecific K2P pairwise sequence divergences for the A) *reference* and B) *novel* data sets (see Table 1 for information on the composition).

FIGURE 6. Agreement between morphologically-grouped specimens based on Linnaean species labels and clusters generated using molecular methods for the *reference* and *novel* data sets. The sample size of species included in each analysis is indicated in parentheses for each data set. The numbers of MOTUs generated are also indicated in parentheses after each analysis method.

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Supporting Information

Additional supporting information may be found in the online version of this article:

gen-2015-0209.R3Supplement 1. Main file (Excel format, with multiple worksheets) containing all data used for calculations, all commands for clustering programs, R script for agreement analysis, and tabulated results from all analyses.

gen-2015-0209.R3Supplement 2. BEAST input XML document for the *novel* data set.

gen-2015-0209.R3Supplement 3. *Novel* data set Bayesian tree results (tree file) from BEAST, used for PTP and GMYC analyses.

gen-2015-0209.R3Supplement 4. *Reference* data set fasta file.

gen-2015-0209.R3Supplement 5. *Novel* data set fasta file.

gen-2015-0209.R3Supplement 6. Methods supplementary file (Word file).

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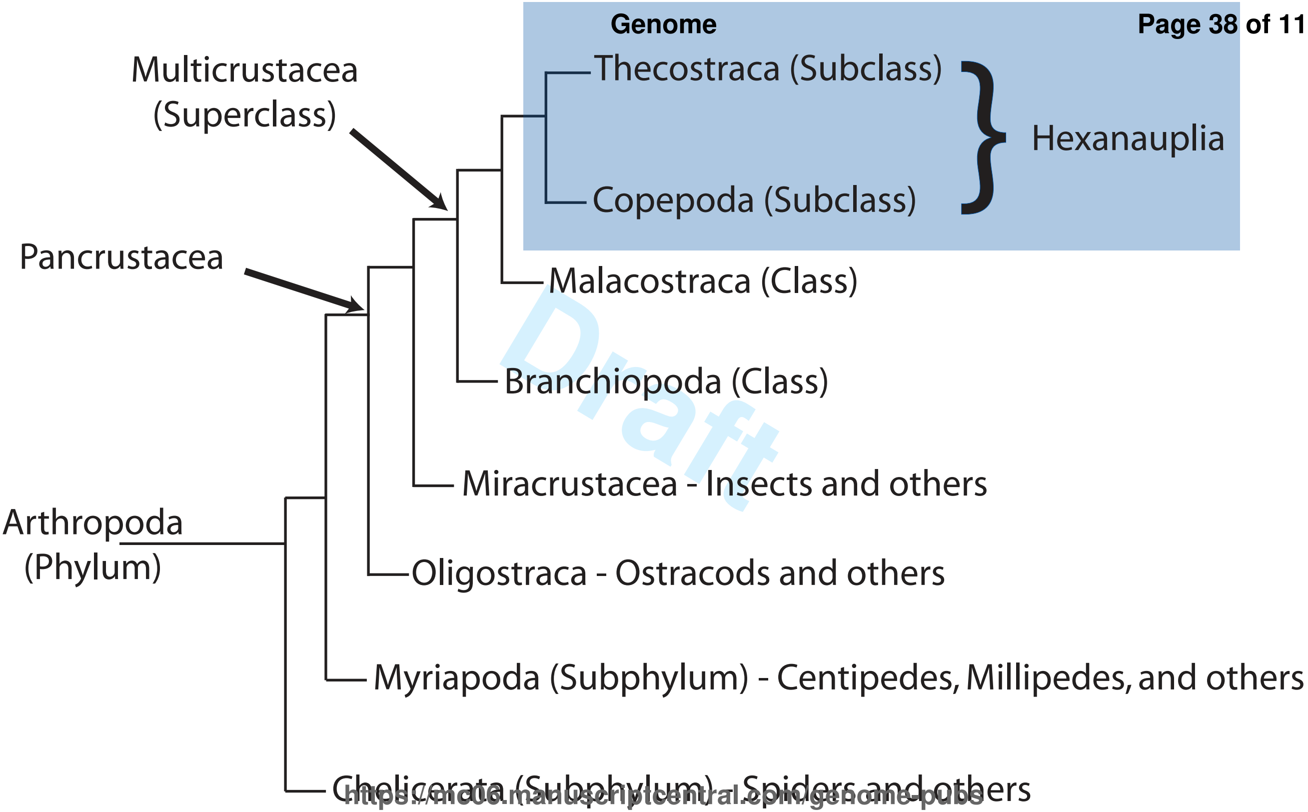

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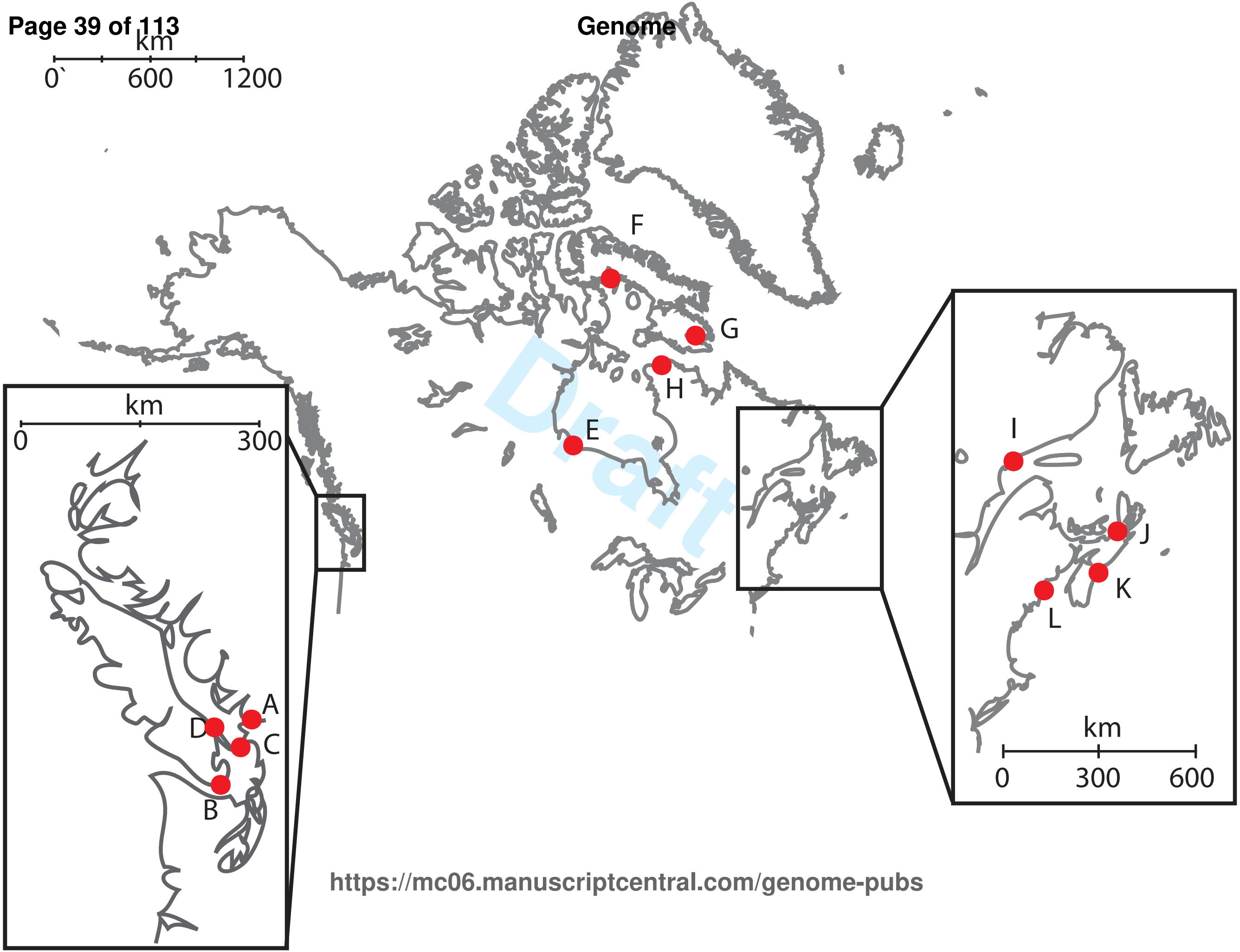
https://mc06.manuscriptcentral.com/genome-pubs



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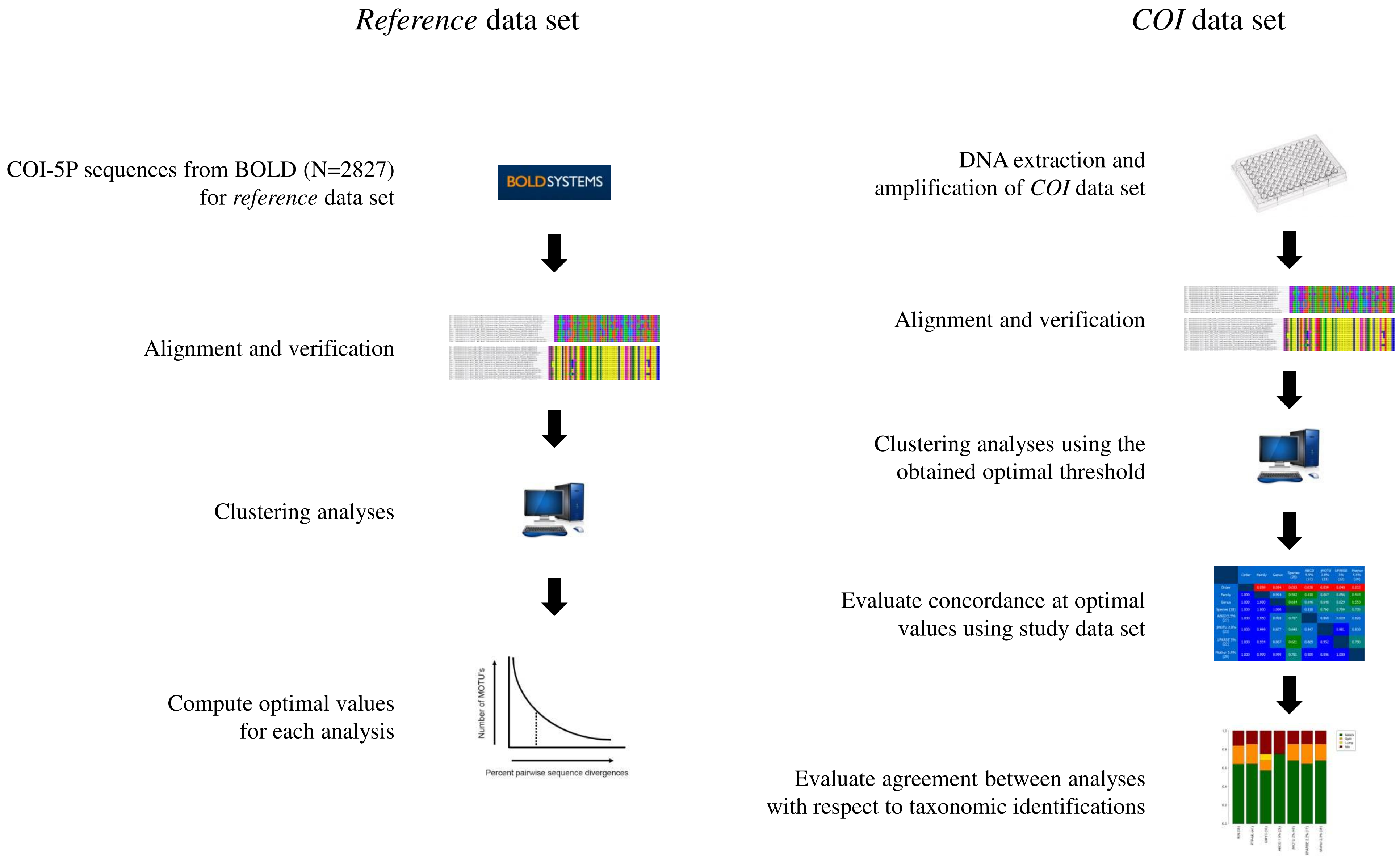


Reference data set

COI-5P sequences from BOLD (N=2827)
for *reference* data set

Alignment and verification

Clustering analyses

Compute optimal values
for each analysis

COI data set

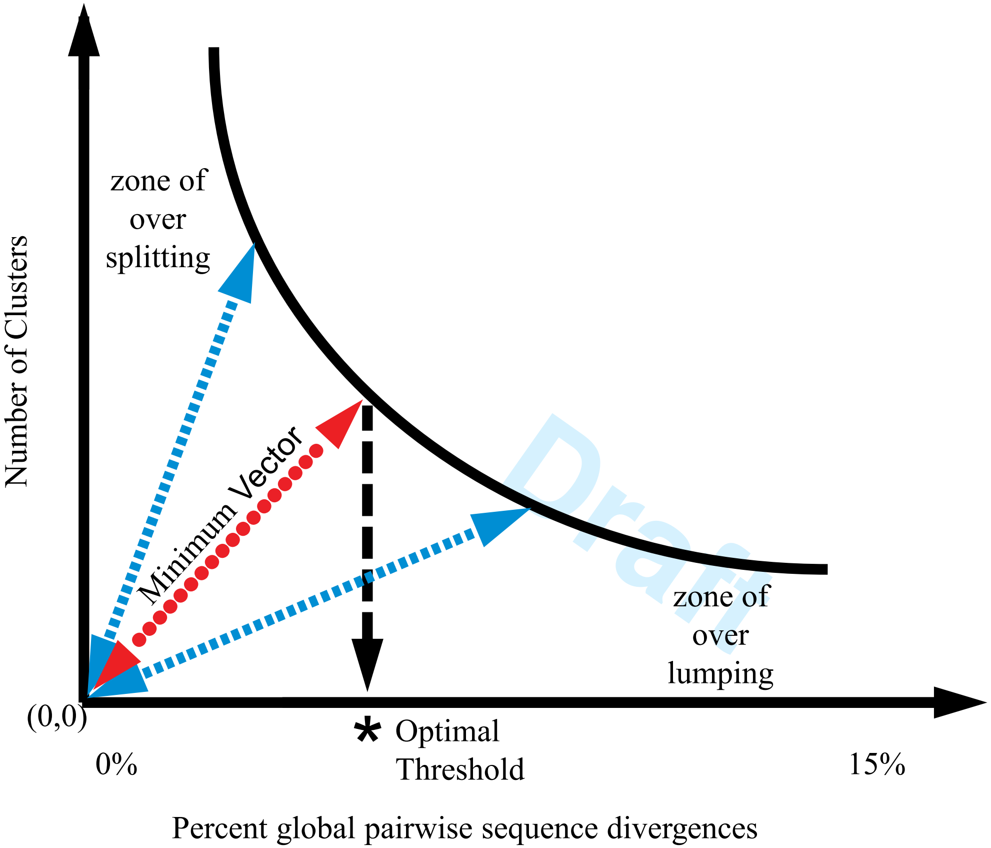
DNA extraction and
amplification of *COI* data set

Alignment and verification

Clustering analyses using the
obtained optimal threshold

Evaluate concordance at optimal
values using study data set

Evaluate agreement between analyses
with respect to taxonomic identifications


zone of
over
splitting
Number of Clusters
Minimum Vector
zone of
over
lumping
(0,0)
* Optimal
Threshold
0%
15%
Percent global pairwise sequence divergences

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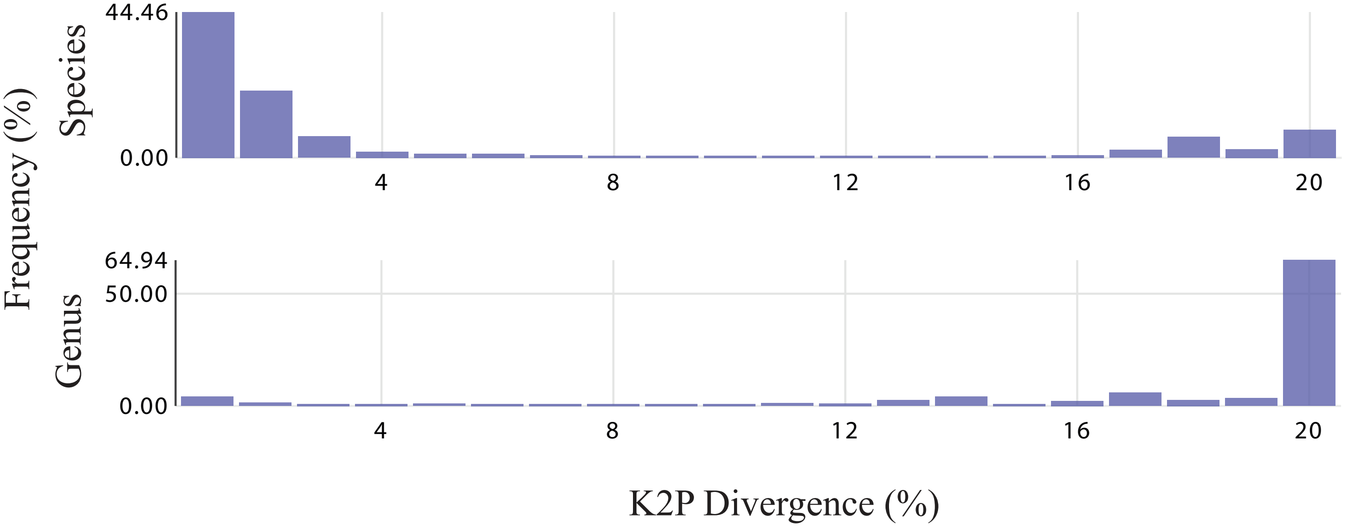
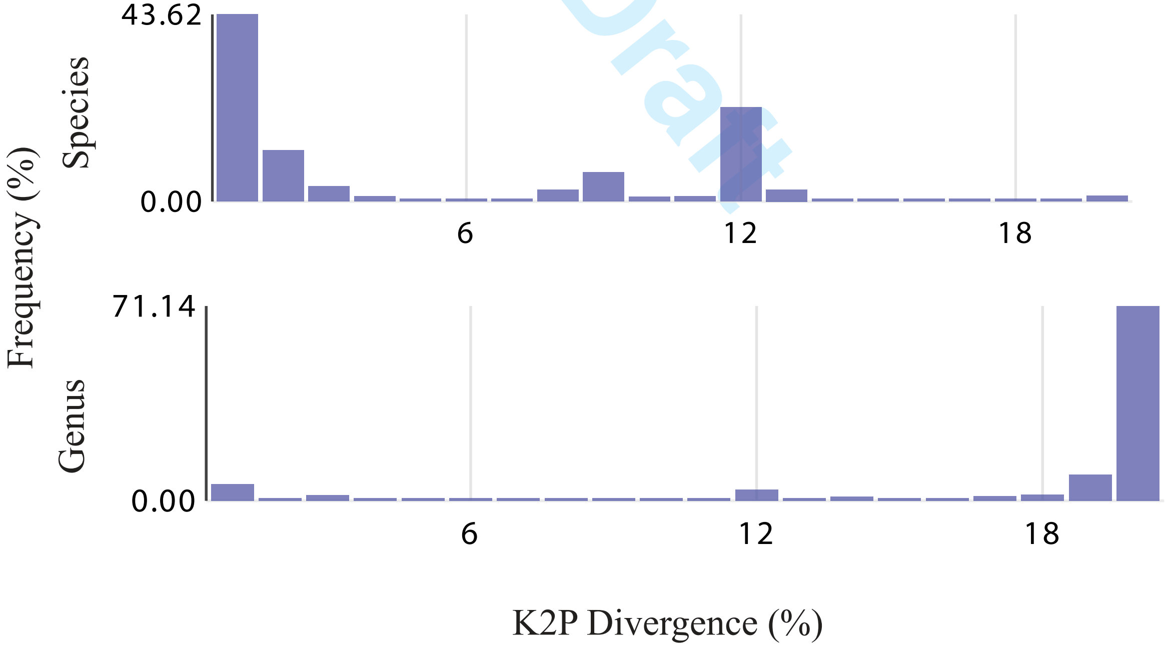
A. *Reference* data set



B. *Novel* data set



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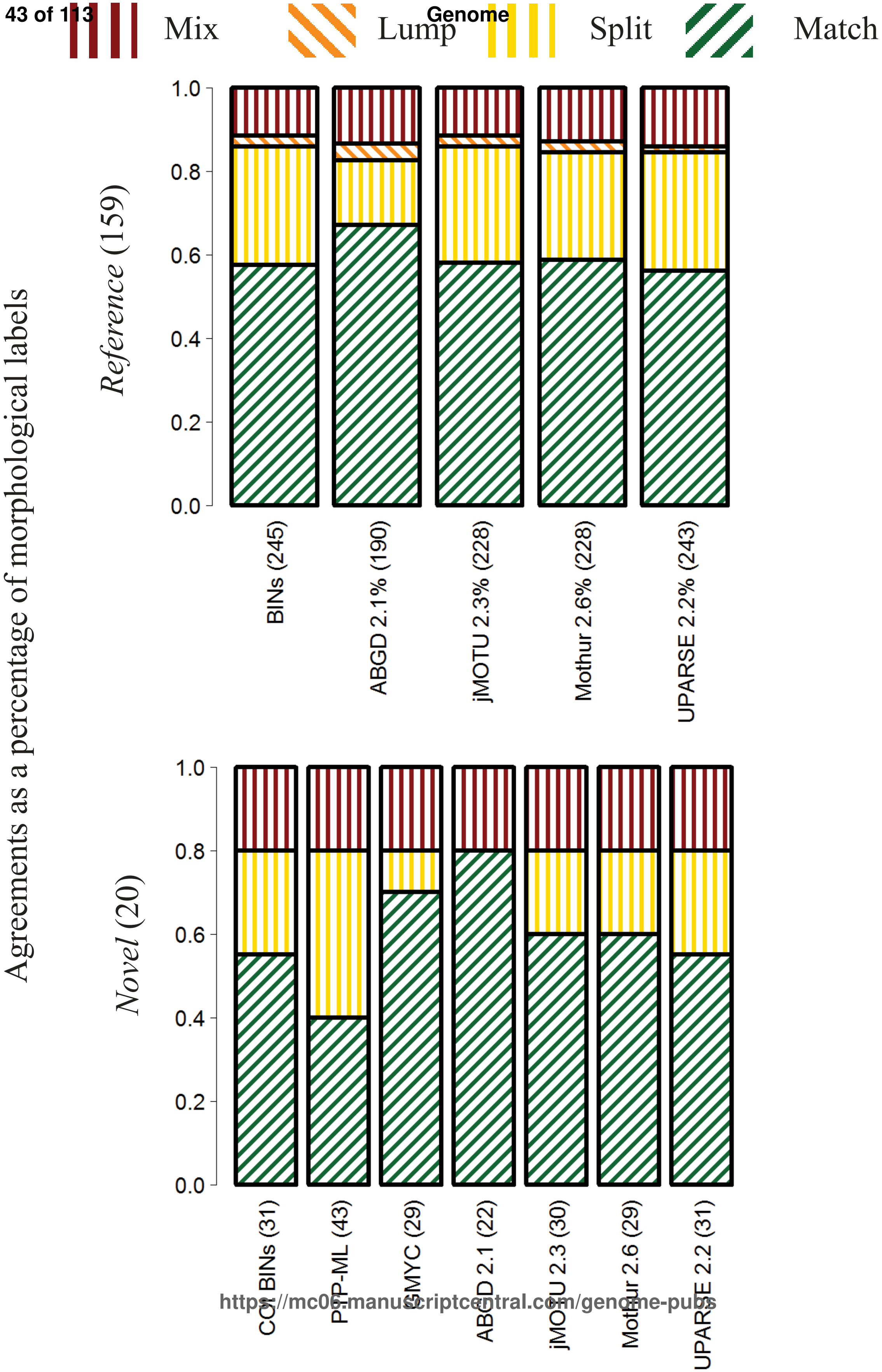
Mix
Lump
Split
Match
Agreements as a percentage of morphological labels
Reference (159)
1.0
0.8
0.6
0.4
0.2
0.0
BINs (245)
ABGD 2.1% (190)
jMOTU 2.3% (228)
Mothur 2.6% (228)
UPARSE 2.2% (243)
Novel (20)
1.0
0.8
0.6
0.4
0.2
0.0
COI BINs (31)
PTP-ML (43)
GMYC (29)
ABGD 2.1 (22)
jMOTU 2.3 (30)
Mothur 2.6 (29)
UPARSE 2.2 (31)

Novel data set results for each of the clustering methods for global pairwise thresholds between 0-3% as wel

Highlighted entries re-added here after the dataset was reduced to unique haplotypes for clustering.	BIN	PTP
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>BIOUG00343-A09_AR_IQT_Calanoida_Acartia_longiremis_BOLD:AAG9533	10	26
>BIOUG00343-A10_AR_IQT_Calanoida_Acartia_longiremis_BOLD:AAG9533	10	26
>BIOUG00343-A11_AR_IQT_Calanoida_Acartia_longiremis_BOLD:AAG9533	10	26
>BIOUG00343-A12_AR_IQT_Calanoida_Acartia_longiremis_BOLD:AAG9533	10	18
>BIOUG00343-B03_AR_IQT_Calanoida_Pseudocalanus_minutus_BOLD:AAE8457	8	9
>BIOUG00343-D04_AR_IQT_Calanoida_Acartia_longiremis_BOLD:AAG9533	10	26
>BIOUG00343-E01_AR_IQT_Calanoida_Calanus_hyperboreus_BOLD:AAD2449	5	7
>BIOUG00343-E12_AR_IQT_Calanoida_Acartia_longiremis_BOLD:AAG9533	10	26
>BIOUG00343-H02_AR_STN_Harpacticoida_Tisbe_furcata_BOLD:ACN6444	40	11
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>BIOUG00343-H07_AR_DBY_Sessilia_Balanus_balanus_BOLD:AAB1410	3	45
>BIOUG00343-H11_AR_CHL_Sessilia_Balanus_balanus_BOLD:AAB1410	3	44
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>BIOUG01717-D02_PA_VIC_Calanoida_Tortanus_discaudatus_BOLD:ACL8332	28	48
>BIOUG01717-D12_PA_VIC_Cyclopoida_Oithona_similis_BOLD:ACK9027	22	51
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>BIOUG04841-B11_AR_DBY_Calanoida_Calanus_glacialis_BOLD:AAM9477	12	24
>BIOUG04841-B12_AR_DBY_Calanoida_Calanus_glacialis_BOLD:AAM9477	12	23
>BIOUG04841-C05_AR_DBY_Harpacticoida_Zaus_abbreviatus_BOLD:ACN4933	39	6
>BIOUG04841-C06_AR_DBY_Harpacticoida_Zaus_abbreviatus_BOLD:ACN3200	37	6
>BIOUG04841-C12_AR_CHL_Sessilia_Balanus_balanus_BOLD:AAB1410	3	44
>BIOUG04841-D03_AR_CHL_Sessilia_Balanus_balanus_BOLD:AAB1410	3	44

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>BIOUG04841-D08_AR_CHL_Calanoida_Tortanus_discaudatus_BOLD:ACL8333	29	4
>BIOUG04841-D09_AR_CHL_Calanoida_Eurytemora_herdmani_BOLD:ACN3121	36	15
>BIOUG04841-D10_AR_CHL_Calanoida_Eurytemora_herdmani_BOLD:AAN6253	13	15
>BIOUG04841-D11_AR_CHL_Calanoida_Eurytemora_herdmani_BOLD:AAN6253	13	15
>BIOUG04841-E05_AR_CHL_Calanoida_Centropages_abdominalis_BOLD:AAD309	6	31
>BIOUG04841-F02_AR_CHL_Calanoida_Acartia_hudsonica_BOLD:AAG9533	10	26
>BIOUG04841-F03_AR_CHL_Calanoida_Acartia_hudsonica_BOLD:AAJ3150	11	41
>BIOUG04841-F10_AR_CHL_Harpacticoida_Microsetella_norvegica_BOLD:ACL797	24	34
>BIOUG04841-G09_AR_STN_Calanoida_Calanus_glacialis_BOLD:AAM9477	12	24
>BIOUG04841-G10_AR_STN_Calanoida_Calanus_glacialis_BOLD:AAM9477	12	24
>BIOUG04841-G11_AR_STN_Calanoida_Calanus_glacialis_BOLD:AAM9477	12	24
>BIOUG04841-G12_AR_STN_Calanoida_Calanus_glacialis_BOLD:AAM9477	12	24
>BIOUG04841-H02_AR_STN_Sessilia_Balanus_balanus_BOLD:AAB1410	3	43
>BIOUG04841-H03_AR_STN_Sessilia_Balanus_balanus_BOLD:AAB1410	3	44
>BIOUG04841-H04_AR_STN_Sessilia_Balanus_balanus_BOLD:AAB1410	3	45
>BIOUG04841-H05_AR_STN_Calanoida_Acartia_longiremis_BOLD:AAG9533	10	26
>BIOUG04841-H06_AR_STN_Calanoida_Acartia_longiremis_BOLD:AAG9533	10	26
>BIOUG04841-H08_AR_STN_Calanoida_Acartia_longiremis_BOLD:AAG9533	10	26
>BIOUG04842-H03_AR_STN_Sessilia_Balanus_balanus_BOLD:AAB1410	3	44
>BIOUG00343-B10_AR_IQT_Calanoida_Acartia_longiremis_BOLD:AAG9533	10	26
>BIOUG00343-E09_AR_IQT_Calanoida_Acartia_hudsonica_BOLD:AAG9533	10	26
>BIOUG00343-E10_AR_IQT_Calanoida_Acartia_hudsonica_BOLD:AAG9533	10	26
>BIOUG00343-E11_AR_IQT_Calanoida_Acartia_longiremis_BOLD:AAG9533	10	26
>BIOUG00343-H08_AR_CHL_Sessilia_Balanus_balanus_BOLD:AAB1410	3	45
>BIOUG01717-D10_PA_VIC_Calanoida_Tortanus_discaudatus_BOLD:ACL8332	28	48
>BIOUG01717-E01_PA_VIC_Calanoida_Acartia_longiremis_BOLD:ACH6220	21	39
>BIOUG01749-B06_PA_VAN_Calanoida_Acartia_longiremis_BOLD:ACH6220	21	39
>BIOUG01749-F04_PA_VAN_Calanoida_Acartia_longiremis_BOLD:ACH6220	21	39
>BIOUG01750-A12_AR_CHL_Calanoida_Paracalanus_parvus_BOLD:ACK9448	23	14
>BIOUG01750-B04_AR_CHL_Sessilia_Balanus_balanus_BOLD:AAB1410	3	45
>BIOUG01750-B05_AR_CHL_Calanoida_Paracalanus_parvus_BOLD:ACK9448	23	14
>BIOUG01750-B07_AR_CHL_Calanoida_Acartia_longiremis_BOLD:AAG9533	10	26
>BIOUG01750-C01_AR_CHL_Calanoida_Skistodiaptomus_pallidus_BOLD:AAB0741	2	13
>BIOUG01750-C03_AR_CHL_Calanoida_Leptodiaptomus_siciloides_BOLD:AAC42	4	12
>BIOUG01750-G02_PA_ROB_Calanoida_Calanus_pacificus_BOLD:AAE8453	7	50
>BIOUG01750-H04_PA_ROB_Calanoida_Acartia_longiremis_BOLD:ACH6220	21	39
>BIOUG01750-H10_PA_VIC_Calanoida_Acartia_longiremis_BOLD:ACH6220	21	39
>BIOUG03170-A01_PA_ROB_Calanoida_Acartia_longiremis_BOLD:ACH6220	21	39
>BIOUG03170-A04_PA_ROB_Calanoida_Acartia_longiremis_BOLD:ACH6220	21	39
>BIOUG03170-A10_PA_ROB_Calanoida_Tortanus_discaudatus_BOLD:ACL8332	28	48
>BIOUG03170-A11_PA_ROB_Calanoida_Tortanus_discaudatus_BOLD:ACL8332	28	48
>BIOUG03170-B06_PA_ROB_Calanoida_Calanus_pacificus_BOLD:AAE8453	7	50
>BIOUG03170-B12_PA_ROB_Calanoida_Centropages_abdominalis_BOLD:AAD30	6	31
>BIOUG03170-E06_PA_NAN_Calanoida_Centropages_abdominalis_BOLD:AAD309	6	31
>BIOUG03170-E09_PA_NAN_Calanoida_Centropages_abdominalis_BOLD:AAD309	6	31
>BIOUG03170-E11_PA_NAN_Calanoida_Paracalanus_parvus_BOLD:ACK9448	23	14
>BIOUG03170-E12_PA_NAN_Calanoida_Paracalanus_parvus_BOLD:ACK9448	23	14
>BIOUG03170-F02_PA_NAN_Calanoida_Acartia_longiremis_BOLD:ACH6220	21	39
>BIOUG03170-F03_PA_NAN_Calanoida_Acartia_longiremis_BOLD:ACH6220	21	39
>BIOUG03170-F05_PA_NAN_Calanoida_Acartia_longiremis_BOLD:ACH6220	21	39
>BIOUG03170-H08_PA_NAN_Calanoida_Calanus_pacificus_BOLD:AAE8453	7	50
>BIOUG03171-F08_PA_ROB_Calanoida_Acartia_longiremis_BOLD:ACH6220	21	39

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>BIOUG03171-F09_PA_ROB_Calanoida_Acartia_longiremis_BOLD:ACH6220	21	39
>BIOUG03171-F11_PA_ROB_Calanoida_Acartia_longiremis_BOLD:ACH6220	21	39
>BIOUG03172-D08_PA_VIC_Calanoida_Acartia_longiremis_BOLD:ACH6220	21	39
>BIOUG03172-D11_PA_VIC_Calanoida_Centropages_abdominalis_BOLD:AAD3099	6	31
>BIOUG03172-E01_PA_VIC_Calanoida_Centropages_abdominalis_BOLD:AAD3099	6	31
>BIOUG03172-E05_PA_VIC_Calanoida_Paracalanus_parvus_BOLD:ACL8030	25	14
>BIOUG03173-C04_PA_VIC_Calanoida_Metridia_pacifica_BOLD:ACL8456	30	3
>BIOUG03173-C07_PA_VAN_Calanoida_Metridia_pacifica_BOLD:ACL8456	30	3
>BIOUG03173-C11_PA_VAN_Calanoida_Paracalanus_parvus_BOLD:ACK9448	23	14
>BIOUG03173-D08_PA_VAN_Calanoida_Calanus_pacificus_BOLD:AAE8453	7	50
>BIOUG03173-D12_PA_VAN_Calanoida_Acartia_longiremis_BOLD:ACH6220	21	39
>BIOUG03173-E02_PA_VAN_Calanoida_Acartia_longiremis_BOLD:ACH6220	21	39
>BIOUG03173-E12_PA_VAN_Calanoida_Centropages_abdominalis_BOLD:AAD309	6	31
>BIOUG03173-F03_PA_VAN_Calanoida_Centropages_abdominalis_BOLD:AAD309	6	31
>BIOUG03179-A09_AL_BAY_Calanoida_Acartia_hudsonica_BOLD:AAJ3150	11	41
>BIOUG03179-B02_AL_BAY_Calanoida_Temora_longicornis_BOLD:AAO2762	14	33
>BIOUG03179-B05_AL_BAY_Calanoida_Temora_longicornis_BOLD:AAO2762	14	32
>BIOUG03179-B08_AL_BAY_Calanoida_Acartia_hudsonica_BOLD:AAJ3150	11	42
>BIOUG04840-B01_AL_HAL_Calanoida_Centropages_hamatus_BOLD:AAW6025	16	30
>BIOUG04840-D10_AL_BSI_Calanoida_Temora_longicornis_BOLD:AAO2762	14	33
>BIOUG04840-D11_AL_BSI_Calanoida_Temora_longicornis_BOLD:AAO2762	14	32
>BIOUG04840-D12_AL_BSI_Calanoida_Temora_longicornis_BOLD:AAO2762	14	32
>BIOUG04840-F03_AL_BSI_Calanoida_Acartia_longiremis_BOLD:AAG9533	10	26
>BIOUG04840-F04_AL_BSI_Calanoida_Acartia_longiremis_BOLD:AAG9533	10	26
>BIOUG04840-F05_AL_BSI_Calanoida_Acartia_longiremis_BOLD:AAG9533	10	26
>BIOUG04840-H06_AL_HAL_Calanoida_Centropages_hamatus_BOLD:AAW6025	16	30
>BIOUG04840-H11_AL_BSI_Calanoida_Acartia_longiremis_BOLD:AAG9533	10	26
>BIOUG04841-A05_AR_DBY_Calanoida_Acartia_longiremis_BOLD:AAG9533	10	26
>BIOUG04841-A07_AR_DBY_Calanoida_Acartia_longiremis_BOLD:AAG9533	10	26
>BIOUG04841-A11_AR_DBY_Harpacticoida_Microsetella_norvegica_BOLD:ACL797	24	34
>BIOUG04841-D12_AR_CHL_Calanoida_Eurytemora_herdmani_BOLD:AAN6253	13	15
>BIOUG04841-E06_AR_CHL_Calanoida_Centropages_abdominalis_BOLD:AAD309	6	31
>BIOUG04841-E07_AR_CHL_Calanoida_Centropages_abdominalis_BOLD:AAD309	6	31
>BIOUG04841-E08_AR_CHL_Calanoida_Centropages_hamatus_BOLD:AAD3099	6	31
>BIOUG04841-F04_AR_CHL_Calanoida_Acartia_hudsonica_BOLD:AAJ3150	11	41
>BIOUG04841-F05_AR_CHL_Calanoida_Acartia_longiremis_BOLD:AAG9533	10	26
>BIOUG04841-F06_AR_CHL_Calanoida_Acartia_longiremis_BOLD:AAG9533	10	26
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>BIOUG04841-F08_AR_CHL_Calanoida_Acartia_longiremis_BOLD:AAG9533	10	26
>BIOUG04841-F11_AR_CHL_Harpacticoida_Microsetella_norvegica_BOLD:ACL797	24	34
>BIOUG04841-H01_AR_STN_Sessilia_Balanus_balanus_BOLD:AAB1410	3	45
>BIOUG04841-H07_AR_STN_Calanoida_Acartia_longiremis_BOLD:AAG9533	10	26
>BIOUG04842-H04_AR_STN_Calanoida_Acartia_longiremis_BOLD:AAG9533	10	26

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l as the Barcode Index Numbers. The numbers are representative of a single cluster as reported by the

GMYC	ABGD 1	UPARSE 1	Mothur 1	jMOTU 1	ABGD 1.1	UPARSE 1	Mothur 1.1	jMOTU 1.1
4	1	1	8	17	1	1	8	17
4	1	1	8	17	1	1	8	17
5	2	2	2	4	2	2	2	4
5	2	2	2	4	2	2	2	4
5	2	2	2	4	2	2	2	4
33	2	2	2	4	2	2	2	4
27	3	3	42	57	3	3	61	50
5	2	2	2	4	2	2	2	4
28	4	4	44	49	4	4	58	44
5	2	2	2	4	2	2	2	4
37	5	5	46	59	5	5	56	52
6	6	6	19	6	6	6	16	6
6	6	6	19	6	6	6	16	6
6	6	7	9	6	6	7	9	6
7	7	8	11	12	7	8	12	12
12	29	9	5	24	29	9	5	23
17	8	10	6	19	8	10	7	19
18	9	11	50	38	9	11	51	35
17	8	10	6	19	8	10	7	19
16	8	10	14	19	8	10	13	19
10	10	12	12	33	10	12	11	16
10	10	13	52	55	10	13	48	48
3	11	14	4	9	11	14	4	9
2	30	15	1	5	30	15	1	5
17	8	10	6	19	8	10	7	19
7	7	8	11	12	7	8	12	12
34	12	16	63	60	12	16	49	53
2	30	15	1	5	30	15	1	5
2	30	15	1	5	30	15	1	5
3	11	14	4	9	11	14	4	9
2	30	15	1	5	30	15	1	5
25	13	17	3	13	13	17	3	13
26	30	18	1	5	30	18	1	5
3	11	14	4	9	11	14	4	9
14	13	17	3	13	13	17	3	13
15	13	19	3	13	13	17	3	13
3	11	20	21	27	11	19	21	25
7	7	21	57	46	7	20	59	42
32	14	22	41	31	14	21	60	29
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15	13	19	3	13	13	23	3	13
15	13	24	24	22	13	24	44	21
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24	15	23	18	15	15	22	17	15
24	15	23	18	15	15	22	17	15
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21	16	25	29	11	16	25	26	11

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12	29	9	5	24	29	9	5	23
16	8	26	14	19	8	26	13	19
17	8	10	6	19	8	10	7	19
10	10	12	34	33	10	12	11	16
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9	17	27	7	10	17	27	6	10
9	17	28	38	53	17	27	31	10
12	29	9	5	24	29	9	5	23
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21	16	29	23	7	16	28	22	7
29	18	30	25	8	18	29	34	8
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19	19	32	13	18	19	31	15	18
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9	17	27	7	10	17	27	6	10
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7	7	8	11	12	7	8	12	12
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22	20	33	16	48	20	32	18	43
22	20	33	16	47	20	32	18	43
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12	29	9	5	24	29	9	5	23
12	29	9	5	24	29	9	5	23
9	17	35	30	40	17	34	35	37
9	17	27	7	10	17	27	6	10
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19	19	32	13	18	19	31	15	18
18	22	36	56	51	22	35	43	46
36	6	37	58	39	6	36	42	36
14	13	17	3	13	13	17	3	13
8	23	38	10	21	23	37	10	20
1	24	39	55	25	24	38	36	24
1	24	40	22	42	24	39	20	39
2	30	15	1	5	30	15	1	5
23	25	41	15	26	25	40	14	1
2	30	42	43	43	30	41	41	5
2	30	15	1	5	30	15	1	5
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8	23	38	10	21	23	37	10	20
8	23	38	10	21	23	37	10	20

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11	26	44	49	37	26	43	38	34
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2	30	15	1	5	30	15	1	5
2	30	15	1	5	30	15	1	5
13	25	45	17	1	25	44	19	1
14	13	17	3	13	13	17	3	13
25	13		26	30	13		23	28
23	33	46	28	50	33	45	24	45
23	25	41	32	26	25	40	14	1
13	25	41	15	26	25	40	14	1
1	24		33	54	24	39	39	47
8	23	38	10	21	23	37	10	20
8	23	38	10	21	23	37	10	20
1	24		27	56	24		37	49
15	13	19	20	13	13	23	3	13
14	13	19	3	13	13	23	3	13
15	13	47	20	13	13	46	3	13
8	23	38	10	21	23	37	10	20
13	25	41	15	26	25	40	14	1
13	25	45	17	1	25	44	19	1
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11	26	51	35	34	26	49	28	31
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3	11	14	4	9	11	14	4	9
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2	30	18	1	5	30	18	1	5
2	30	15	1	5	30	15	1	5
1	24	40	22	42	24	39	20	39
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4	1	54	60	17	1	52	55	17
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12	29	9	5	24	29	9	5	23
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9	17	27	7	10	17	27	6	10
9	17	27	7	10	17	27	6	10
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12	29	9	5	24	29	9	5	23
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10	10	12	12	33	10	12	11	16
12	29	9	5	24	29	9	5	23

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9	17	27	7	10	17	27	6	10
9	17	27	7	10	17	27	6	10
7	7	21	57	46	7	20	59	42
35	21	34	39	41	21	33	40	38
35	21	34	39	41	21	33	40	38
7	7	8	11	12	7	8	12	12
10	10	12	12	33	10	12	11	16
12	29	9	5	24	29	9	5	23
12	29	9	5	24	29	9	5	23
9	17	27	7	10	17	27	6	10
9	17	27	7	10	17	27	6	10
13	25	45	17	1	25	44	19	1
1	24		33	54	24	39	39	47
1	24	40	22	42	24	39	20	39
23	25	41	15	26	25	40	14	1
8	23	38	10	21	23	37	10	20
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1	24	40	22	42	24	39	20	39
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5	2	2	2	4	2	2	2	4
8	23	38	10	21	23	37	10	20
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3	11	14	4	9	11	14	4	9
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9	17	27	7	10	17	27	6	10
13	25	45	17	1	25	44	19	1
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5	2	2	2	4	2	2	2	4
5	2	2	2	4	2	2	2	4
3	11	14	4	9	11	14	4	9
6	6	6	19	6	6	6	16	6
5	2	2	2	4	2	2	2	4
5	2	2	2	4	2	2	2	4

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cooresponding analysis. All samples (as indicated by the fasta headers within column A) with the same

ABGD 1.2	UPARSE 1	Mothur 1.2	jMOTU 1.2	ABGD 1.3	UPARSE 1	Mothur 1.3	jMOTU 1.3	ABGD 1.4
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2	2	2	4	2	2	2	4	2
2	2	2	4	2	2	2	4	2
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4	4	49	42	4	4	43	42	4
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5	5	47	49	5	5	44	49	5
6	6	19	5	6	6	19	5	6
6	6	19	5	6	6	19	5	6
6	7	9	5	6	7	9	5	6
7	8	12	11	7	8	12	11	7
29	9	6	21	29	9	8	21	29
8	10	7	18	8	10	4	18	8
9	11	51	33	9	11	45	33	9
8	10	7	18	8	10	4	18	8
8	10	14	18	8	10	4	18	8
10	12	11	15	10	12	11	15	10
10	13	53	45	10	13	47	45	10
11	14	4	8	11	14	5	8	11
8	15	1	3	8	15	1	3	8
8	10	7	18	8	10	4	18	8
7	8	12	11	7	8	12	11	7
12	16	48	50	12	16	51	50	12
8	15	1	3	8	15	1	3	8
8	15	1	3	8	15	1	3	8
11	14	4	8	11	14	5	8	11
8	15	1	3	8	15	1	3	8
13	17	3	12	13	17	3	12	13
8	15	1	3	8	15	1	3	8
11	14	4	8	11	14	5	8	11
13	17	3	12	13	17	3	12	13
13	17	3	12	13	17	3	12	13
11	18	22	23	11	18	21	23	11
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8	10	7	18	8	10	4	18	8
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7	8	12	11	7	8	12	11	7
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13	17	3	12	13	17	3	12	13
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24	37	16	37	24	37	15	37	24
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25	38	13	1	25	38	13	1	25
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8	15	1	3	8	15	1	3	8
13	22	3	12	13	22	3	12	13
23	35	10	19	23	35	10	19	23
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25	38	13	1	25	38	13	1	25
24	37	16	37	24	37	15	37	24
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23	35	10	19	23	35	10	19	23
24	37	26	46	24	37	31	46	24
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13	22	3	12	13	22	3	12	13
13	43	3	12	13	43	3	12	13
23	35	10	19	23	35	10	19	23
25	38	13	1	25	38	13	1	25
25	41	18	1	25	41	17	1	25
25	44	24	2	25	44	25	2	25
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8	15	25	3	8	15	23	3	8
8	15	1	3	8	15	1	3	8
27	45	23	25	27	45	22	25	27
26	46	27	29	26	46	24	29	26
11	14	4	8	11	14	5	8	11
11	14	4	8	11	14	5	8	11
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8	15	1	3	8	15	1	3	8
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28	49	43	39	28	49	53	39	28
28		44	24	28		52	24	28
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17	26	5	9	17	26	7	9	17
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15	21	17	14	15	21	16	14	15
10	13	53	45	10	13	47	45	10
29	9	6	21	29	9	8	21	29
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29	9	6	21	29	9	8	21	29

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7	8	12	11	7	8	12	11	7
10	12	11	15	10	12	11	15	10
29	9	6	21	29	9	8	21	29
29	9	6	21	29	9	8	21	29
17	26	5	9	17	26	7	9	17
17	26	5	9	17	26	7	9	17
25	41	18	1	25	41	17	1	25
24	37	16	37	24	37	15	37	24
24	37	16	37	24	37	15	37	24
25	38	13	1	25	38	13	1	25
23	35	10	19	23	35	10	19	23
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24	37	16	37	24	37	15	37	24
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23	35	10	19	23	35	10	19	23
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13	22	3	12	13	22	3	12	13
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17	26	5	9	17	26	7	9	17
25	41	18	1	25	41	17	1	25
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2	2	2	4	2	2	2	4	2
11	14	4	8	11	14	5	8	11
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number for a single analysis column were clustered in the same group.

UPARSE 1	Mothur 1.4	jMOTU 1.4	ABGD 1.5	UPARSE 1	Mothur 1.5	jMOTU 1.5	ABGD 1.6	UPARSE 1
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1	7	16	1	1	7	16	1	1
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2	2	4	2	2	2	4	2	2
2	2	4	2	2	2	4	2	2
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4	43	40	4	4	49	40	4	4
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5	40	46	5	5	47	46	5	5
6	5	5	6	6	5	5	6	6
6	5	5	6	6	5	5	6	6
7	5	5	6	7	5	5	6	6
8	12	11	7	8	12	11	7	7
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12	11	15	10	12	11	15	10	11
13	52	43	10	13	39	43	10	12
14	6	8	11	14	6	8	11	13
15	1	3	8	15	1	3	8	14
10	4	18	8	10	4	18	8	9
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22	21	10	16	22	26	10	16	21

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35	14	1	25	35	15	1	25	34
35	14	1	25	35	15	1	25	34
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40	24	24	27	40	28	24	27	39
41	22	28	26	41	27	28	26	40
14	6	8	11	14	6	8	11	13
14	6	8	11	14	6	8	11	13
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42	28	27	30	42	21	27	30	41
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Draft

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Draft

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Draft

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Draft

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Draft

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Draft

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Draft

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Draft

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Draft

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Draft

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Draft

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Draft

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Draft

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Draft

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Draft

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Draft

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29	8	9	19
29	8	9	19
10	11	11	13
29	8	9	19

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29	8	9	19
29	8	9	19
29	8	9	19
17	20	8	9
17	20	8	9
7	16	24	29
21	24	29	28
21	24	29	28
7	7	14	10
10	11	11	13
29	8	9	19
29	8	9	19
17	20	8	9
17	20	8	9
25	28	10	1
24	27	13	20
24	27	13	20
25	28	10	1
23	26	12	17
24	27	13	20
24	27	13	20
24	27	13	20
2	2	2	4
2	2	2	4
2	2	2	4
23	26	12	17
2	2	2	4
2	2	2	4
2	2	2	4
11	12	4	8
13	15	3	11
17	20	8	9
17	20	8	9
17	20	8	9
25	28	10	1
2	2	2	4
2	2	2	4
2	2	2	4
2	2	2	4
11	12	4	8
6	6	6	5
2	2	2	4
2	2	2	4

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Novel data set clustering outcomes and the patterns of association (or agreements) with morphological

Morphological ID Linnean name	COI BIN's	PTP	GMYC	ABGD 1	ABGD 1.1	ABGD 1.2
Acartia clausi	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
Acartia hudsonica	MIX	MIX	MIX	MIX	MIX	MIX
Acartia longiremis	MIX	MIX	MIX	MIX	MIX	MIX
Aetideus divergens	MATCH	SPLIT	MATCH	MATCH	MATCH	MATCH
Balanus balanus	LUMP	SPLIT	MATCH	LUMP	LUMP	LUMP
Balanus glandula	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
Calanus glacialis	MATCH	SPLIT	MATCH	MATCH	MATCH	MATCH
Calanus hyperboreus	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
Calanus pacificus	MATCH	SPLIT	MATCH	MATCH	MATCH	MATCH
Centropages abdominalis	MIX	MIX	MIX	MIX	MIX	MIX
Centropages hamatus	MIX	MIX	MIX	MIX	MIX	MIX
Centropages typicus	SPLIT	SPLIT	MATCH	MATCH	MATCH	MATCH
Corycaeus anglicus	LUMP	MATCH	MATCH	LUMP	LUMP	LUMP
Epilabidocera amphitrites	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
Eurytemora herdmani	MIX	MIX	MIX	MIX	MIX	MIX
Leptodiaptomus siciloides	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
Mesocalanus tenuicornis	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
Metridia pacifica	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
Microsetella norvegica	MATCH	SPLIT	MATCH	MATCH	MATCH	MATCH
Monstrilla scotti	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
Oithona atlantica	MATCH	MATCH	LUMP	MATCH	MATCH	MATCH
Oithona similis	MATCH	MATCH	LUMP	MATCH	MATCH	MATCH
Paracalanus parvus	SPLIT	SPLIT	MATCH	MATCH	MATCH	MATCH
Pseudocalanus minutus	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
Skistodiaptomus pallidus	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
Temora longicornis	MIX	MIX	MIX	MIX	MIX	MIX
Tisbe furcata	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
Tortanus discaudatus	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	MATCH
Zaus abbreviatus	SPLIT	SPLIT	MATCH	MATCH	MATCH	MATCH

MATCH	17	14	20	20	20	21
SPLIT	4	9	1	1	1	0
LUMP	2	0	2	2	2	2
MIX	6	6	6	6	6	6
TOTAL	29	29	29	29	29	29

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species identifications.

ABGD 1.3	ABGD 1.4	ABGD 1.5	ABGD 1.6	ABGD 1.7	ABGD 1.8	ABGD 1.9	ABGD 2	ABGD 2.1
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
MIX	MIX	MIX	MIX	MIX	MIX	MIX	MIX	MIX
MIX	MIX	MIX	MIX	MIX	MIX	MIX	MIX	MIX
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
LUMP	LUMP	LUMP	LUMP	LUMP	LUMP	LUMP	LUMP	LUMP
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
MIX	MIX	MIX	MIX	MIX	MIX	MIX	MIX	MIX
MIX	MIX	MIX	MIX	MIX	MIX	MIX	MIX	MIX
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
LUMP	LUMP	LUMP	LUMP	LUMP	LUMP	LUMP	LUMP	LUMP
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
MIX	MIX	MIX	MIX	MIX	MIX	MIX	MIX	MIX
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
MIX	MIX	MIX	MIX	MIX	MIX	MIX	MIX	MIX
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
21	21	21	21	21	21	21	21	21
0	0	0	0	0	0	0	0	0
2	2	2	2	2	2	2	2	2
6	6	6	6	6	6	6	6	6
29	29	29	29	29	29	29	29	29

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ABGD 2.2	ABGD 2.3	ABGD 2.4	ABGD 2.5	ABGD 2.6	ABGD 2.7	ABGD 2.8	ABGD 2.9	ABGD 3
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
MIX	MIX	MIX	MIX	MIX	MIX	MIX	MIX	MIX
MIX	MIX	MIX	MIX	MIX	MIX	MIX	MIX	MIX
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
LUMP	LUMP	LUMP	LUMP	LUMP	LUMP	LUMP	LUMP	LUMP
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
MIX	MIX	MIX	MIX	MIX	MIX	MIX	MIX	MIX
MIX	MIX	MIX	MIX	MIX	MIX	MIX	MIX	MIX
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
LUMP	LUMP	LUMP	LUMP	LUMP	LUMP	LUMP	LUMP	LUMP
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
MIX	MIX	MIX	MIX	MIX	MIX	MIX	MIX	MIX
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
MIX	MIX	MIX	MIX	MIX	MIX	MIX	MIX	MIX
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH

21	21	21	21	21	21	21	21	21
0	0	0	0	0	0	0	0	0
2	2	2	2	2	2	2	2	2
6	6	6	6	6	6	6	6	6
29	29	29	29	29	29	29	29	29

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jMOTU 1	jMOTU 1.1	jMOTU 1.2	jMOTU 1.3	jMOTU 1.4	jMOTU 1.5	jMOTU 1.6	jMOTU 1.7	jMOTU 1.8
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
MIX	MIX	MIX	MIX	MIX	MIX	MIX	MIX	MIX
MIX	MIX	MIX	MIX	MIX	MIX	MIX	MIX	MIX
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	MATCH	MATCH	MATCH
SPLIT	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT
MIX	MIX	MIX	MIX	MIX	MIX	MIX	MIX	MIX
MIX	MIX	MIX	MIX	MIX	MIX	MIX	MIX	MIX
SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
SPLIT	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
SPLIT	SPLIT	SPLIT	SPLIT	MATCH	MATCH	MATCH	MATCH	MATCH
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT
SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT

14	16	16	16	17	17	18	18	18
11	9	9	9	8	8	7	7	7
0	0	0	0	0	0	0	0	0
4	4	4	4	4	4	4	4	4
29	29	29	29	29	29	29	29	29

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jMOTU 1.9	jMOTU 2	jMOTU 2.1	jMOTU 2.2	jMOTU 2.3	jMOTU 2.4	jMOTU 2.5	jMOTU 2.6	jMOTU 2.7
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
MIX	MIX	MIX	MIX	MIX	MIX	MIX	MIX	MIX
MIX	MIX	MIX	MIX	MIX	MIX	MIX	MIX	MIX
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
MATCH	MATCH	LUMP	LUMP	LUMP	LUMP	LUMP	LUMP	LUMP
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
SPLIT	SPLIT	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
MIX	MIX	MIX	MIX	MIX	MIX	MIX	MIX	MIX
MIX	MIX	MIX	MIX	MIX	MIX	MIX	MIX	MIX
SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT
MATCH	MATCH	LUMP	LUMP	LUMP	LUMP	LUMP	LUMP	LUMP
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	MIX	MIX
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	MIX	MIX
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT
SPLIT	SPLIT	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
18	18	18	18	18	18	18	18	18
7	7	5	5	5	5	5	3	3
0	0	2	2	2	2	2	2	2
4	4	4	4	4	4	4	6	6
29	29	29	29	29	29	29	29	29

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jMOTU 2.8	jMOTU 2.9	jMOTU 3	Mothur 1	Mothur 1.1	Mothur 1.2	Mothur 1.3	Mothur 1.4	Mothur 1.5
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
MIX	MIX	MIX	MIX	MIX	MIX	MIX	MIX	MIX
MIX	MIX	MIX	MIX	MIX	MIX	MIX	MIX	MIX
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
LUMP	LUMP	LUMP	SPLIT	SPLIT	SPLIT	SPLIT	MATCH	MATCH
MATCH	MATCH	MATCH	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT
MATCH	MATCH	MATCH	SPLIT	SPLIT	SPLIT	MATCH	MATCH	MATCH
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
MATCH	MATCH	MATCH	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT
MIX	MIX	MIX	MIX	MIX	MIX	MIX	MIX	MIX
MIX	MIX	MIX	MIX	MIX	MIX	MIX	MIX	MIX
SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT
LUMP	LUMP	LUMP	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
MIX	MIX	MIX	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
MATCH	MATCH	MATCH	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
MIX	MIX	MIX	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT
MATCH	MATCH	MATCH	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT
18	18	18	14	14	14	15	16	16
3	3	3	11	11	11	10	9	9
2	2	2	0	0	0	0	0	0
6	6	6	4	4	4	4	4	4
29	29	29	29	29	29	29	29	29

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Mothur 1.6	Mothur 1.7	Mothur 1.8	Mothur 1.9	Mothur 2	Mothur 2.1	Mothur 2.2	Mothur 2.3	Mothur 2.4
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
MIX	MIX	MIX	MIX	MIX	MIX	MIX	MIX	MIX
MIX	MIX	MIX	MIX	MIX	MIX	MIX	MIX	MIX
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	LUMP
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	MATCH	MATCH
MIX	MIX	MIX	MIX	MIX	MIX	MIX	MIX	MIX
MIX	MIX	MIX	MIX	MIX	MIX	MIX	MIX	MIX
SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	LUMP
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
SPLIT	SPLIT	SPLIT	SPLIT	MATCH	MATCH	MATCH	MATCH	MATCH
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT
SPLIT	SPLIT	SPLIT	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH

17	17	17	18	19	19	19	20	18
8	8	8	7	6	6	6	5	5
0	0	0	0	0	0	0	0	2
4	4	4	4	4	4	4	4	4
29	29	29	29	29	29	29	29	29

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Mothur 2.5	Mothur 2.6	Mothur 2.7	Mothur 2.8	Mothur 2.9	Mothur 3	UPARSE 1	UPARSE 1	UPARSE 1
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
MIX	MIX	MIX	MIX	MIX	MIX	MIX	MIX	MIX
MIX	MIX	MIX	MIX	MIX	MIX	MIX	MIX	MIX
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
LUMP	LUMP	LUMP	LUMP	LUMP	LUMP	SPLIT	SPLIT	SPLIT
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	SPLIT	SPLIT	SPLIT
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	SPLIT	SPLIT	SPLIT
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	SPLIT	SPLIT	SPLIT
MIX	MIX	MIX	MIX	MIX	MIX	MIX	MIX	MIX
MIX	MIX	MIX	MIX	MIX	MIX	MIX	MIX	MIX
SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT
LUMP	LUMP	LUMP	LUMP	LUMP	LUMP	MATCH	MATCH	MATCH
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	SPLIT	SPLIT	SPLIT
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	SPLIT	SPLIT	SPLIT

18	18	18	18	18	18	14	14	14
5	5	5	5	5	5	11	11	11
2	2	2	2	2	2	0	0	0
4	4	4	4	4	4	4	4	4
29	29	29	29	29	29	29	29	29

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UPARSE 1	UPARSE 1	UPARSE 1	UPARSE 1	UPARSE 1	UPARSE 1	UPARSE 1	UPARSE 2	UPARSE 2
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
MIX	MIX	MIX	MIX	MIX	MIX	MIX	MIX	MIX
MIX	MIX	MIX	MIX	MIX	MIX	MIX	MIX	MIX
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
SPLIT	SPLIT	SPLIT	MATCH	MATCH	MATCH	MATCH	MATCH	LUMP
SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	MATCH	MATCH	MATCH	MATCH
SPLIT	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	MATCH	MATCH	MATCH
MIX	MIX	MIX	MIX	MIX	MIX	MIX	MIX	MIX
MIX	MIX	MIX	MIX	MIX	MIX	MIX	MIX	MIX
SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	LUMP
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
SPLIT	SPLIT	SPLIT	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT
SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	MATCH	MATCH	MATCH
14	15	15	17	17	18	20	20	18
11	10	10	8	8	7	5	5	5
0	0	0	0	0	0	0	0	2
4	4	4	4	4	4	4	4	4
29	29	29	29	29	29	29	29	29

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UPARSE 2	UPARSE 2	UPARSE 2	UPARSE 2	UPARSE 2	UPARSE 2	UPARSE 2	UPARSE 2	UPARSE 3
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
MIX	MIX	MIX	MIX	MIX	MIX	MIX	MIX	MIX
MIX	MIX	MIX	MIX	MIX	MIX	MIX	MIX	MIX
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
LUMP	LUMP	LUMP	LUMP	LUMP	LUMP	LUMP	LUMP	LUMP
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
MIX	MIX	MIX	MIX	MIX	MIX	MIX	MIX	MIX
MIX	MIX	MIX	MIX	MIX	MIX	MIX	MIX	MIX
SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT
LUMP	LUMP	LUMP	LUMP	LUMP	LUMP	LUMP	LUMP	LUMP
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
SPLIT	SPLIT	MIX	MIX	MIX	MIX	MIX	MIX	MIX
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
SPLIT	SPLIT	MIX	MIX	MIX	MIX	MIX	MIX	MIX
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT	SPLIT
MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH	MATCH
18	18	18	18	18	18	18	18	18
5	5	3	3	3	3	3	3	3
2	2	2	2	2	2	2	2	2
4	4	6	6	6	6	6	6	6
29	29	29	29	29	29	29	29	29

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Methods supplementary file S6

Molecular laboratory methodology

DNA extraction followed Ivanova et al. (2006) with six variations: (1) a CTAB lysis solution (2% CTAB, 100mM Tris-HCL, pH 8.0, 20mM EDTA, pH8.0, 1.4M NaCl) was used in place of the indicated Vertebrate Lysis Buffer; (2) a 1 mm^3 piece of specimen was used; (3) a 1.2X dilution (using ddH_2O) of binding buffer was used; (4) after addition of binding buffer, the total solution was incubated on the bench top for 5 min; (5) a 2X dilution of protein wash buffer was used; and (6) final DNA extracts were eluted in 50 µl of ddH_2O.

Multiple PCR primer sets were used to amplify the animal barcode region. The predominant primer set used was LCO1490 / HCO2198 (Folmer et al. 1994). Additional primer sets used are listed here in order of amplification success: (1) Folmer et al. (1994) primers tailed with M13 (Messing 1983) (LCO1490_t1 / HCO2198_t1); (2) a zooplankton-specific primer pair (ZplankF1_t1 / ZplankR1_t1) (Prosser et al. 2013); (3) a degenerate primer set based on the Folmer primers (dgLCO1490 / dgHCO2198) (Meyer 2003); (4) a Lepidoptera and Folmer primer cocktail (C_LepFolF / C_LepFolR, 1:1) (Folmer et al. 1994; Hajibabaei et al. 2006); and (5) a crustacean primer set (CrustDF1 - GGTCWACAAAYCATAAAGAYATTGG, CrustDR1 - TAAACYTCAGGRTGACCRAARAAYCA) (Steinke unpublished). Initial PCRs were processed in 96-well plates following the Canadian Centre for DNA Barcoding (CCDB) protocols (Ivanova and Grainger 2007a). A second PCR protocol was used when necessary and had a final chemistry of 2 mM $MgCl_2$, 0.2 mM dNTP's, 0.4 µM forward and reverse primer, 1X PCR buffer, 10% DNA template by volume, and 0.0064 Units of Taq/µl in final volume. The thermocycling regime used for all reactions was: 95°C for 5min; 40 cycles of 95°C for 40sec, 50°C for 40sec, 72°C for 60sec; 72°C for 10min. PCR products were visualized using either a

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1.5% agarose gel or a bufferless E-gel system (Life Technologies). PCR products were cleaned using EXOSap-IT. Bidirectional sequencing followed CCDB sequencing protocols using Big Dye 3.1 (Ivanova and Grainger 2007b).

CodonCode Aligner (CodonCode Corporation) was used to display sequence quality and assemble consensus sequences. All unassembled chromatograms and consensus sequences of less than half the expected length (~650 base pairs [bp]) were visually inspected and removed if the quality of the chromatograms and subsequent base calling was poor.

Screening data for potential contaminations and/or misidentifications

Neighbour-joining (NJ) phenograms (Saitou and Nei 1987) using Kimura-2-parameter (K2P) distances (Kimura 1980) were constructed through MEGA6 and tested using 10,000 bootstrap pseudoreplicates. The K2P model was chosen due to its prevalence in the barcoding literature, enabling comparisons across studies; p-distances, although advocated by some authors (Srivathsan and Meier 2012; Collins et al. 2012), vs. K2P are expected to behave similarly at small genetic distances (Srivathsan and Meier 2012; Collins et al. 2012). Phenograms were visually inspected for potential contaminants; non-target DNA could be sequenced as a result of DNA present in the shared ethanol storage medium, because of preferential binding of primers to a symbiont or prey item, or due to trace contamination during laboratory procedures. Two possible scenarios resulted in the removal of sequences: (1) a search of the COI-5P barcode data on BOLD's public data portal returned a result where there was 100% placement probability to a class other than Maxillopoda (mismatches were verified using the tree-based identification tool in BOLD to guard against matches to incorrectly identified sequences in the database); and (2) where three or more individuals were morphologically identified as being the same species,

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sequences that displayed more than 20% divergence (Blanco-Bercial et al. 2014) from others were deleted due to presumed contamination or misidentification (re-identifications for individual specimens, e.g. as in Renaud et al. 2012, were precluded by the consumptive DNA analysis). Specimens morphologically identified as the same genus or species by the same identifier and from the same sample, but which grouped more closely with another genus, were flagged as potential contaminants and saved for further verification using additional information, as they may have indicated biologically significant variation, such as cryptic species or hybridization.

Bayesian tree construction

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The Bayesian tree was constructed with the Bayesian Evolutionary Analysis Sampling Trees (BEAST) (Drummond et al. 2012) program using the GTR+G+I model and a log-normal relaxed clock with rate estimation, as a strict clock is less likely to apply across the large and diverse multicrustacean groups studied. The tree prior was set to the Yule process, and the initial ucld.mean value was set between 0 and 10. Five independent runs of 100,000,000 Markov Chain Monte Carlo (MCMC) generations were conducted, with sampling every 1000 generations. Results for each of these runs were inspected using the Tracer program (Rambaut et al. 2014), with convergence visually verified. All five runs were combined using the BEAST program LogCombiner, where a 10% burn-in was applied (first 10% of trees eliminated), and 45,000 states for each MCMC run were subsampled from each run. The BEAST program TreeAnnotator was then used to summarize the LogCombined tree file into a single target tree by finding the best-fit tree using the specified maximum clade credibility type tree.

References

Blanco-Bercial, L., Cornils, A., Copley, N., and Bucklin, A. 2014. DNA barcoding of marine copepods: assessment of analytical approaches in species identification. *PLoS Curr,* **6**: 1-21. doi:10.1371/currents.tol.cdf8b74881f87e3b01d56b43791626d2 PMCID: PMC 4073882

Collins, R.A., Boykin, L.M., Cruickshank, R.H., and Armstrong, K.F. 2012. Barcoding's next top model: an evaluation of nucleotide substitution models for specimen identification. *Meth. Ecol. Evol.*, **3**(3): 457–465. doi: 10.1111/j.2041-210x.2011.00176.x

Drummond, A.J., Suchard, M.A., Xie, D., and Rambaut, A. 2012. Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Mol. Biol. Evol.*, **29**(8): 1969–1973. doi:10.1093/molbev/mss075

Folmer, O., Black, M., Hoeh, W., Lutz, R., and Vrijenhoek, R. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Mol. Mar. Biol. Biotechnol.*, **3**(5): 294–299.

Hajibabaei, M., Janzen, D.H., Burns, J.M., Hallwachs, W., and Hebert, P.D.N. 2006. DNA barcodes distinguish species of tropical Lepidoptera. *Proc. Natl. Acad. Sci. U.S.A.*, **103**(4): 968–971. doi: 10.1073/pnas.0510466103

Ivanova, N.V., deWaard, J.R., and Hebert, P.D.N. 2006. An inexpensive, automation–friendly protocol for recovering high-quality DNA. *Mol. Ecol. Notes,* **6**: 998–1002. doi: 10.1111/j.1471- 8286.2007.01748.x

Ivanova, N.V., and Grainger, C.M. 2007a. CCDB Protocols, COI amplification. http://ccdb.ca//CCDB_DOCS/CCDB_Amplification.pdf.

Ivanova, N.V., Grainger, C.M. 2007b. CCDB Protocols, COI sequencing. http://ccdb.ca//CCDB_DOCS/CCDB_Sequencing.pdf.

Kimura, M. 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J. Mol. Evol.*, **16**(2): 111–120.

Knowlton, N. 1993. Sibling species in the sea. *Annu. Rev. Ecol. Syst.*, **24**: 189–216.

Messing, J. 1983. New M13 vectors for cloning. *Meth. Enzymol.,* **101**: 20–78.

Meyer, C.P. 2003. Molecular systematics of cowries (Gastropoda: Cypraeidae) and diversification patterns in the tropics. *Biol. J. Linn. Soc. Lond.*, **79**(3): 401–459.

Prosser, S., Martínez-Arce, A., and Elías-Gutiérrez, M. 2013. A new set of primers for COI amplification from freshwater microcrustaceans. *Mol. Ecol. Resour.*, **13**(6): 1151–1155. doi: 10.1111/1755-0998.12132

Draft

Rambaut, A., Suchard, M.A., Xie, D., and Drummond, A.J. 2014. Tracer v1.6, Available from http://beast.bio.ed.ac.uk/Tracer

Renaud, A.K., Savage, J., and Adamowicz, S.J. 2012. DNA barcoding of Northern Nearctic Muscidae (Diptera) reveals high correspondence between morphological and molecular species limits. *BMC Ecol.*, **12**(1): 24. doi: 10.1186/1472-6785-12-24

Saitou, N., and Nei, M. 1987. The Neighbor-joining Method: A New Method for Reconstructing Phylogenetic Trees. *Mol. Biol. Evol.*, **4**(4): 406–425.

Srivathsan, A., and Meier, R. 2012. On the inappropriate use of Kimura-2-parameter (K2P) divergences in the DNA-barcoding literature. *Cladistics*, **28**(2): 190–194. doi:10.1111/j.1096-0031.2011.00370.x

Draft