Biological identifications through DNA barcodes: the case of the Crustacea

Filipe O. Costa, Jeremy R. deWaard, James Boutillier, Sujeevan Ratnasingham, Robert T. Dooh, Mehrdad Hajibabaei, and Paul D.N. Hebert

Abstract: The ability of a 650 base pair section of the mitochondrial cytochrome *c* oxidase I (COI) gene to provide species-level identifications has been demonstrated for large taxonomic assemblages of animals such as insects, birds, and fishes, but not for the subphylum Crustacea, one of the most diverse groups of arthropods. In this study, we test the ability of COI to provide identifications in this group, examining two disparate levels in the taxonomic hierarchy — orders and species. The first phase of our study involved the development of a sequence profile for 23 dominant crustacean orders, based upon the analysis of 150 species, each belonging to a different family. The COI amino acid data placed these taxa into cohesive assemblages whose membership coincided with currently accepted boundaries at the order, superorder, and subclass levels. Species-level resolution was subsequently examined in an assemblage of Decapoda and in representatives of the genera *Daphnia* (Cladocera) and *Gammarus* (Amphipoda). These studies revealed that levels of nucleotide sequence divergence were from 19 to 48 times greater between congeneric species than between individuals of a species. We conclude that sequence variation in the COI barcode region will be very effective for discriminating species of Crustacea.

Résumé : Il a été démontré que l'utilisation d'une section de 650 paires de bases du gène mitochondrial de la cytochrome *c* oxydase I (COI) permet de faire des identifications au niveau spécifique de grands ensembles taxonomiques d'animaux tels que les insectes, les oiseaux et les poissons, mais pas encore dans le sous-phylum des crustacés, l'un des groupes les plus diversifiés d'arthropodes. Nous testons dans notre étude le potentiel de l'utilisation de COI pour faire des identifications dans ce groupe en examinant deux niveaux disparates de la hiérarchie taxonomique — les ordres et les espèces. La première phase de notre recherche consiste en l'établissement de profils de séquences pour 23 des ordres principaux de crustacés, d'après l'analyse de 150 espèces, appartenant chacune à une famille différente. Les données sur les acides aminés de COI permettent de placer ces taxons en ensembles cohésifs dont la composition coïncide avec les frontières couramment acceptées aux niveaux de l'ordre, du super-ordre et de la sous-classe. Nous avons ensuite étudié la détermination au niveau spécifique chez un ensemble de décapodes et chez des représentants des genres *Daphnia* (Cladocera) et *Gammarus* (Amphipoda). Ces études révèlent que les niveaux de divergence des séquences de nucléotides sont de 19 à 48 fois plus importants entre les espèces d'un même genre qu'entre les individus d'une même espèce. Nous concluons que la variation des séquences dans la région du code-barre de COI devrait permettre de séparer de façon très efficace les espèces de crustacés.

[Traduit par la Rédaction]

Introduction

The ideal DNA-based identification system would employ a single gene for the placement of any organism in the full taxonomic hierarchy from kingdom to species. The ability of a ~658 base pair (bp) section of the mitochondrial cytochrome *c* oxidase I (COI) gene to provide this resolution has now been demonstrated for many animal lineages, including lepidopterans (Hebert et al. 2003; Janzen et al. 2005; Hajibabaei et al. 2006), birds (Hebert et al. 2004), spiders (Barrett and Hebert 2005), ants (Smith et al. 2005), and fishes (Ward et al. 2005) (but see Dasmahapatra and Mallet 2006 for a discussion of success rates). Although COI appears to have high potential as the foundation for an identification system, it is critical to verify that it can deliver similar taxonomic resolution in other groups. The present study addresses this issue by examining the patterning of COI diversity in the subphylum Crustacea, the most ancient and structurally diverse group of arthropods. The diversity of modern crustaceans, in addition to their remarkable vari-

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F.O. Costa.[1,2] Instituto do Mar (IMAR), Universidade Nova de Lisboa, 2829-516 Caparica, Portugal.
J.R. deWaard, S. Ratnasingham, R.T. Dooh, M. Hajibabaei, and P.D.N. Hebert. Biodiversity Institute of Ontario, University of Guelph, ON N1G 2W1, Canada.
J. Boutillier. Pacific Biological Station, Fisheries and Oceans Canada, 3190 Hammond Bay Road, Nanaimo, BC V9T 6N7, Canada.

[1]Corresponding author (e-mail: f.o.costa@bangor.ac.uk).
[2]Present address: School of Biological Sciences, University of Wales, Bangor, Bangor, Gwynedd, LL57 2UW, United Kingdom.

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ety in morphologies, habitats, and ecologies, is reflected by their assignment to six classes, 48 orders, 849 different families, and about 52 000 species (Martin and Davis 2001). By comparison, the more than one million described species of insects are assigned to about 1000 families, but just 30 orders and a single class (Borror et al. 1989; Nielsen 1995).

While the higher level systematics (i.e., class, subclass) of the Crustacea are not fully stabilized (Martin and Davis 2001), ordinal boundaries have been nearly constant for more than 20 years despite ongoing morphological and molecular studies. As such, this taxonomic rank represents a useful criterion to judge the ability of a DNA-based identification system to deliver coarse taxonomic placement of an unknown taxon. However, as the primary task of taxonomy lies in identification of species, it is also critical to test the effectiveness of DNA barcodes at this level. The present study examines the effectiveness of a 658 bp section near the 5′ end of COI (hereafter referred to as the COI barcode) as an identification tool at these two disparate levels in the taxonomic hierarchy — orders and species. Its effectiveness at the ordinal level was tested in a two-step process: 150 species, each in a different family and including representatives from 23 of the 48 crustacean orders, were sequenced to establish a COI ordinal profile. The effectiveness of this profile in ordinal-level placements was subsequently evaluated by determining its success in the assignment of 100 new taxa to the proper order. The present study also sought to determine if the COI barcode region can enable species-level assignments within the Crustacea; this issue was examined in representatives of nine genera of Decapoda and in single genera of Cladocera and Amphipoda.

Materials and methods

Taxon sampling

COI ordinal profile

One hundred and fifty species, each belonging to a different family and including representatives from 23 of 48 crustacean orders, were sequenced to establish a COI ordinal profile (sequences for 75 of these families were acquired from GenBank, see below). More than 10 families were examined from each of four orders (Amphipoda, Calanoida, Decapoda, Isopoda), between four and nine families from eight other orders (Anostraca, Cladocera, Cumacea, Diplostraca, Harpacticoida, Podocopida, Sessilia, Stomatopoda), while one to two families were examined from the other 11 orders. Sequences were also obtained for 100 additional species (53 were acquired from GenBank, see below) in families included in the profile, but belonging to different genera to provide a group of test taxa (details on specimens are provided in Appendix A, Tables A1 and A2).

Decapod case study

Family- and species-level resolution was analyzed in a data set that comprised members of the order Decapoda. Most newly analyzed decapods were collected on research cruises led by Fisheries and Oceans Canada (DFO) off the Pacific coast of Canada in 2002 and 2004, but a few species were collected in Resolute Bay, Nunavut; in the Gulf of Gdansk, Poland; and off Lizard Island, Australia. These specimens were identified to the species level after collection and were subsequently preserved in 100% ethanol. In total, sequences were newly gathered for 146 specimens, representing 57 species, and these results were merged with 173 sequences from GenBank, representing 71 species (complete list of decapods in Appendix A, Tables A3 and A4).

Selected crustacean genera

To obtain detailed information on the patterning of COI diversity among closely related Crustacea, additional data were assembled on two genera to enable a comparison of congeneric versus conspecific divergences. This analysis was performed for 13 species in the cladoceran genus *Daphnia* (Adamowicz et al. 2004) and for 12 species in the amphipod genus *Gammarus* (F.O. Costa and P.D.N. Hebert, unpublished data). A complete list of species used in these analyses is provided in Appendix A, Tables A5 and A6.

GenBank data

With more than 5000 entries, COI is the most intensively studied crustacean gene, representing nearly 50% of the GenBank mitochondrial DNA data for this subphylum (GenBank search, 30 June 2005). Although these entries provide COI sequences for over 600 species, very few include a complete sequence for the gene. Because the partial sequences were acquired with varied primers, the opportunities for sequence comparisons are limited. From sequences available in GenBank (September 2004), 1585 providing coverage for the COI barcode region were extracted. Use of these data was further limited by the short lengths of some sequences. Only those with a minimum of 500 bp of the COI barcode were used, with a single exception to accommodate a small group of 498 bp sequences for the crab *Carcinus* (Roman and Palumbi 2004). The data from GenBank do provide valuable information for analyses that require a broad taxonomic coverage, such as an examination of shifts in guanine–cytosine (GC) usage among crustacean lineages. For this purpose, a total of 617 sequences were extracted and analyzed at various taxonomic levels.

DNA extraction, amplification, and sequencing

In the case of newly analyzed specimens for the ordinal study, DNA was extracted using the proteinase K protocol (Schwenk et al. 1998) or Isoquick (Orca Research Inc., Bothell, Washington). A 658 bp fragment from the 5′ end of COI was amplified using the primer pair LCO1490 (5′-GGTCAACAAATCATAAAGATATTGG-3′) and HCO2198 (5′-TAAACTTCAGGGTGACCAAAAAATCA-3′) (Folmer et al. 1994). Each polymerase chain reaction (PCR) had a total volume of 50 μL and contained 5 μL of 10× PCR buffer, 1.5 mmol·L^{-1} of $MgCl_2$, 50 μmol·L^{-1} of each dNTP, 1 unit (U) of *Taq* polymerase (1 U ≈ 16.67 nkat), 0.3 μmol·L^{-1} of each primer, and 2–4 μL of DNA template. The PCR thermal regime consisted of 60 s at 94 °C; five cycles of 30 s at 94 °C, 90 s at 45 °C, and 60 s at 72 °C; 35 cycles of 30 s at 94 °C, 90 s at 51 °C, and 60 s at 72 °C; followed by a final extension of 5 min at 72 °C. The PCR product was subsequently gel-purified with Qiaex II kit (Qiagen Inc., Valencia, California.) and sequenced in one direction on an ABI 377

automated sequencer (Applied Biosystems, Inc., Foster City, California).

A slightly different protocol was used in the decapod case study; DNA was isolated using either the GenElute kit (Sigma Inc., St. Louis, Missouri) or the Chelex dry release method (Hajibabaei et al. 2005). The COI barcode was amplified using HCO2198 as reverse primer combined with one of three forward primers: LCO1490, CrustF1 (5′-TTTTCTACAAATCATAAAGACATTGG-3′), or CrustF2 (5′-GGTTCTTCTCCACCAACCACAARGAYATHGG-3′). Each polymerase chain reaction had a total volume of 50 µL and contained 5 µL of 10× PCR buffer, 2.2 mmol·L^{-1} of $MgCl_2$, 50 µmol·L^{-1} of each dNTP, 1.5 U of *Taq* polymerase, 0.2 µmol·L^{-1} of each primer, and 1 µL of DNA template. The PCR thermal regime was identical to the one described above except for the CrustF2 primer, which was as follows: 1 cycle of 60 s at 94 °C; 35 cycles of 30 s at 94 °C, 90 s at 42 °C, and 60 s at 72 °C; followed by a final cycle of 5 min at 72 °C. The PCR product was subsequently gel-purified with UltraClean™15 DNA purification kit (MoBio Laboratories, Inc., Solana Beach, California) and sequenced in one or both directions using an ABI 377 or ABI 3730. For sequencing reactions we used BigDye® Terminator v3.1 cycle sequencing kit (Applied Biosystems, Inc., Foster City, California).

Data analysis

Sequence data, electropherograms, and primer details for each newly analyzed specimen are available in the completed project file entitled "Crustacean Barcodes" on the Barcode of Life Data Systems (BOLD) website (http://www.barcodinglife.org). COI sequences were edited using SeqApp 1.9 sequence editor (http://iubio.bio.indiana.edu/soft/molbio/seqapp/) and Sequencher (Gene codes Corporation, Ann Arbor, Michigan) and aligned using CLUSTAL W (Thompson et al. 1994) implemented in MEGA3 (Kumar et al. 2004). To accommodate GenBank data, all sequences were subsequently pruned to 658 bp.

Neighbor-joining (NJ) analysis, implemented in MEGA3 (Kumar et al. 2004), was employed for graphical representation of the patterns of COI divergences among crustacean species. The Kimura two-parameter (K2P) distance model was used to calculate nucleotide divergences (Kimura 1980), while Poisson correction (Nei and Kumar 2000) was used to compute amino acid divergences for the ordinal profile. Both the ordinal amino acid NJ tree and the Decapod nucleotide NJ tree were subjected to 10 000 bootstraps.

The first phase of the ordinal analysis involved the generation of an NJ tree for the 150 profile taxa. This profile was subsequently used as a classification engine by rerunning the NJ analysis following repeated addition of a single test taxon to the data set. After each analysis, the test species was assigned membership in the same taxonomic group as its nearest neighboring taxon. For example, a test taxon was identified as a member of the order Anostraca if it grouped most closely with any one of the seven anostracan families included in the profile. The success of the classification was quantified by determining the proportion of test taxa assigned to their proper order.

Levels of COI nucleotide divergences among conspecific and congeneric individuals were compared in *Daphnia*, *Gammarus*, and in those genera from the decapod data set that included at least three species and two specimens of each species. For this purpose, the "Distance Summary" implemented in BOLD (Ratnasingham and Hebert 2007) was used. This feature performs an automated computation of pairwise divergences at the species, genus, and family level for all possible specimen combinations in the data set. The average values obtained for conspecific and congeneric divergences were applied in the calculation of the taxonomic resolution ratio, which is defined as the quotient between congeneric divergences and conspecific divergences.

Results

Ordinal profile

The amino acid NJ tree for representatives of 150 crustacean families showed high cohesion of taxonomic groups. Most allied species formed assemblages that mirrored established higher-taxonomic categories — orders, superorders, or subclasses (Fig. 1; a detailed tree can be found in Supplemental Appendix S1).[3] For example, members of the orders Sessilia and Pedunculata formed a cohesive group as superorder Thoracica, while members of the subclasses Copepoda and Phyllopoda formed distinctive assemblages. Members of the orders Amphipoda, Anostraca, Isopoda, Mysida, and Stomatopoda were also joined in cohesive clusters. There were, however, a few deviations from this pattern; members of the order Decapoda split into two groups, with Stomatopoda embedded within. As well, two species in the orders Cumacea and Amphipoda (*Pseudocuma similis* and *Themisto gaudichaudii*, respectively) were genetically divergent from allied taxa, grouping instead with species from orders represented by a single family. A few other species, such as the parasitic isopods, (*Bopyroides hippolytes*, *Olencira praegustator*, *Rocinela angustata*), occupied isolated positions in the tree.

The ordinal profile was subsequently used to classify 100 newly analyzed species. In 95 of these cases, the test taxon was assigned to the correct order. In three cases, the test taxa were assigned only at a rank above the order level, namely *Drepanopus* sp., *Eurycercus longirostris*, and *Mytilocypris ambiguosa*, which were assigned to subclasses Copepoda and Phyllopoda and class Ostracoda, respectively. Two misassignments involved taxa in the order Mysidacea, namely *Paramesopodopsis rufa* and *Tenagomysis australis*.

Decapod study

Members of most (13 of 17) decapod families represented by more than one species formed a cohesive cluster. However, species in the families Palaemonidae, Portunidae, and Oplophoridae were each split into two separate clusters, while Paguridae clustered with Lithodidae. The NJ tree for 127 species of Decapoda did, however, reveal the regular

[3] Supplementary data for this article are available on the journal Web site (http://cjfas.nrc.ca) or may be purchased from the Depository of Unpublished Data, Document Delivery, CISTI, National Research Council Canada, Building M-55, 1200 Montreal Road, Ottawa, ON K1A 0R6, Canada. DUD 5134. For more information on obtaining material refer to http://cisti-icist.nrc-cnrc.gc.ca/irm/unpub_e.shtml.

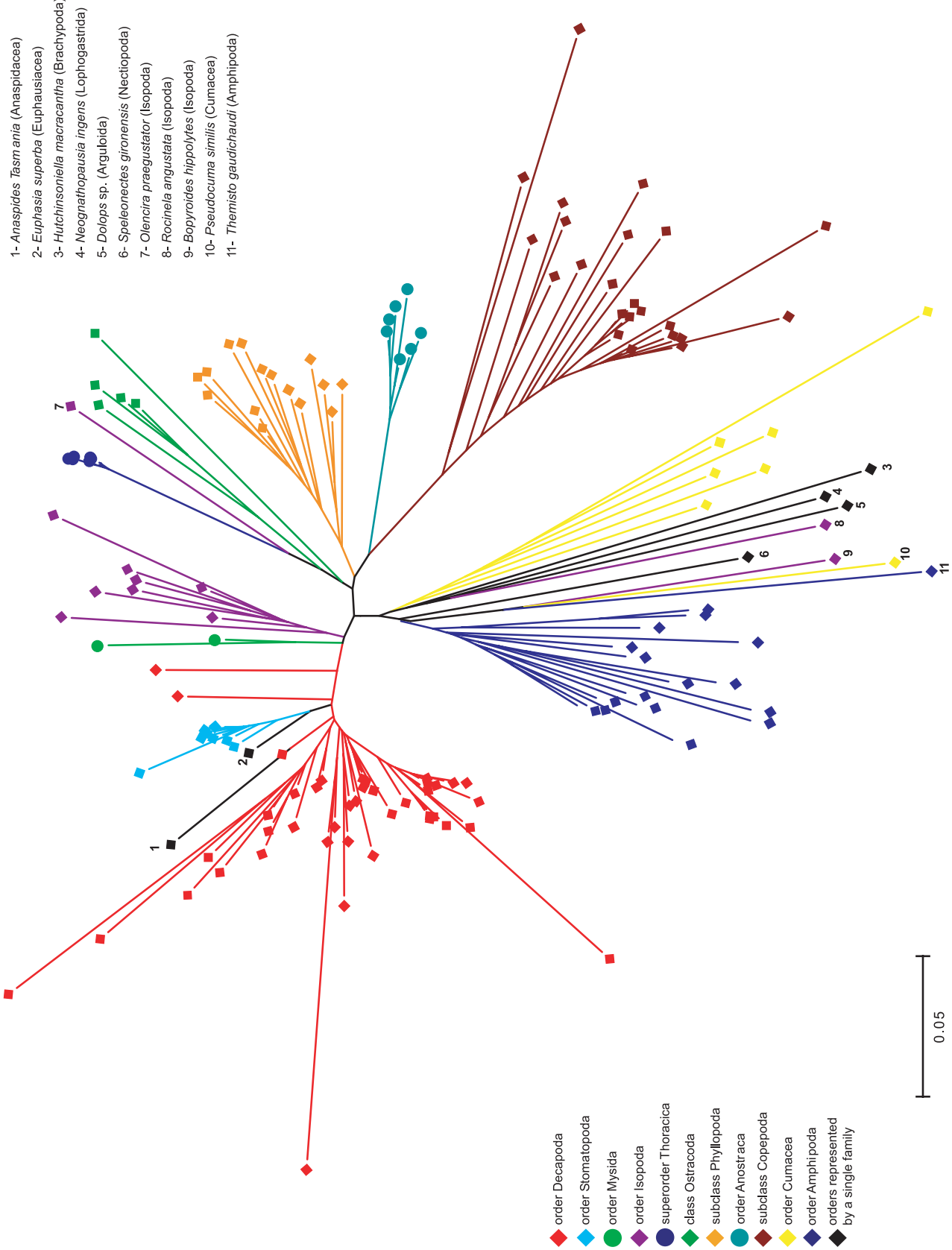
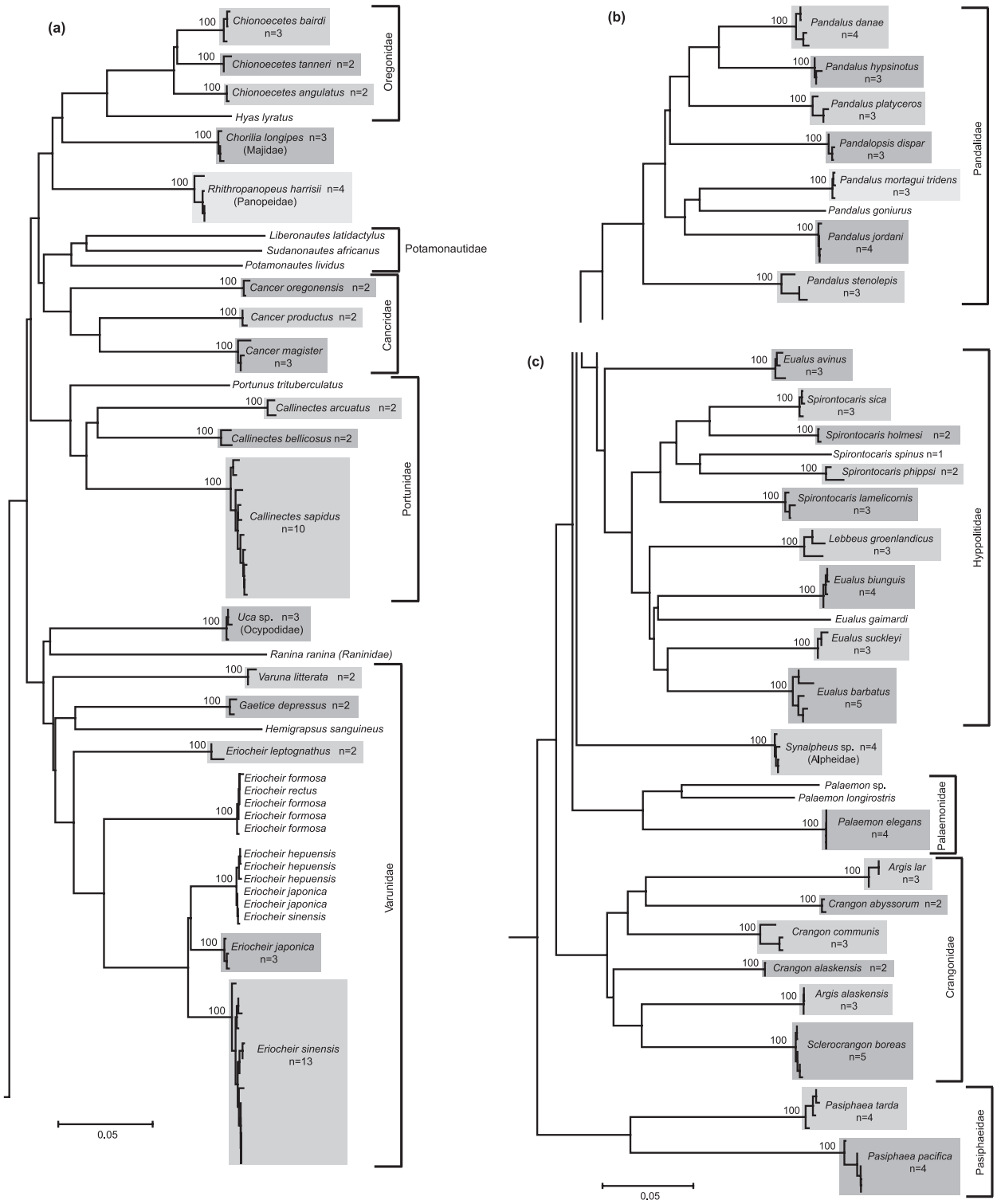


Fig. 1. Neighbor-joining radiation tree showing mitochondrial cytochrome *c* oxidase I (COI) barcode amino acid profile for 150 crustacean species, each belonging to a different family. Numbers 1–11 indicate species from orders represented here by a single family, as well as species divergent from allied taxa. The detailed tree is displayed in Supplemental Appendix S1.[3]

Fig. 2. Three illustrative branches of the neighbor-joining tree based on mitochondrial cytochrome *c* oxidase I (COI) barcode nucleotide distances (K2P) for 127 decapod species representing 30 families. Bootstrap values for 10 000 replicates are shown near the respective branches. The complete tree is displayed in Supplemental Appendix S2.[3]



ability of COI sequences to provide species-level resolution (Fig. 2; the complete tree can be found in Supplemental Appendix S2).[3] COI sequences formed assemblages congruent with known species boundaries for all taxa from the Pacific Coast of Canada, Resolute Bay, and the Baltic Sea. We had comparable results with GenBank sequences (correct assignment = 93.0%), with only 5 congeneric taxa of unsettled species status not being discriminated with the COI sequences. All five taxa belong to the mitten crab genus *Eriocheir*, where species-level taxonomy has been problematic

Table 1. Pairwise mitochondrial cytochrome *c* oxidase I (COI) barcode nucleotide divergences for Decapoda and selected crustacean genera, using K2P distances (%).

Taxon[a]	Pairwise divergences	No. of comparisons	Min. distance	Mean distance[b]	Max. distance	TRR[c]	Reference
Order Decapoda	Within a species	144	0.00	0.46±0.05	2.57	37.6	This study
(54 species, 31 genera,	Within a genus	409	4.92	17.16±0.18	23.66		
138 sequences)	Within a family	459	11.27	19.75±0.17	49.93		
	Within a order	8441	15.30	27.65±0.05	49.00		
Genus *Cancer*	Within a species	5	0.16	0.50±0.15	0.97	34.6	This study
(3 species, 7 sequences)	Within a genus	16	14.97	17.24±0.45	20.01		
Genus *Chionoecetes*	Within a species	5	0.00	0.26±0.08	0.47	21.5	This study
(3 species, 7 sequences)	Within a genus	16	4.92	5.67±0.09	6.28		
Genus *Crangon*	Within a species	5	0.00	0.94±0.41	2.13	20.1	This study
(3 species, 7 sequences)	Within a genus	16	17.31	18.91±0.41	21.74		
Genus *Eualus*	Within a species	22	0.00	0.59±0.14	2.57	32.5	This study
(5 species, 16 sequences)	Within a genus	98	15.17	19.24±0.23	23.64		
Genus *Pandalus*	Within a species	24	0.00	0.48±0.11	1.86	34.4	This study
(6 species, 21 sequences)	Within a genus	186	9.85	16.49±0.18	20.82		
Genus *Spirontocaris*	Within a species	8	0.15	0.47±0.11	1.20	32.9	This study
(5 species, 11 sequences)	Within a genus	47	10.80	15.40±0.29	18.74		
GenBank Decapoda							
Genus *Callinectes*	Within a species	47	0.11	0.57±0.03	1.08	29.9	Pfeiler et al. 2005
(3 species, 14 sequences)	Within a genus	44	15.86	17.03±0.10	18.09		Darden et al., unpublished data[d]
Genus *Cherax*	Within a species	22	0.17	2.81±0.52	9.12	5.7	Munasinghe et al. 2003
(3 species, 14 sequences)	Within a genus	69	10.43	15.95±0.42	22.63		Miller et al. 2004
Genus *Raymunida*	Within a species	26	0.00	0.25±0.08	2.17	48.3	Macpherson and Machordom 2001
(4 species, 17 sequences)	Within a genus	110	7.6	12.08±0.21	16.41		
Other crustaceans							
Order Cladocera							
Genus *Daphnia*	Within a species	472	0.00	1.32±0.05	4.30	19.2	Adamowicz et al. 2004
(13 species, 80 sequences)	Within a genus	2688	13.18	25.28±0.05	30.65		
Order Amphipoda							
Genus *Gammarus*	Within a species	284	0	0.74±0.05	3.09	26.1	Costa and Hebert, unpublished data[e]
(12 species, 69 sequences)	Within a genus	2062	5.58	25.33±0.06	31.39		

[a]Number of species with more than one sequence and number of sequences analysed are shown in parentheses.
[b]Data reported as K2P distance (%) ± SE.
[c]Taxonomic resolution ratio (see Data analysis in Materials and methods).
[d]R.L. Darden, B.R. Kreiser, and A. Place, Biological Sciences, University of Southern Mississippi, 118 College Drive #5018, Hattiesburg, MS 39406, USA.
[e]F.O. Costa and P.D.N. Hebert, School of Biological Sciences, University of Wales, Bangor, Bangor, Gwynedd, LL57 2UW, United Kingdom.

(Chu et al. 2003), and its members show incomplete resolution in the NJ tree with *E. formosa* and *E. rectus* in one cluster and *E. hepuensis*, *E. sinensis*, and *E. japonica* in a second cluster.

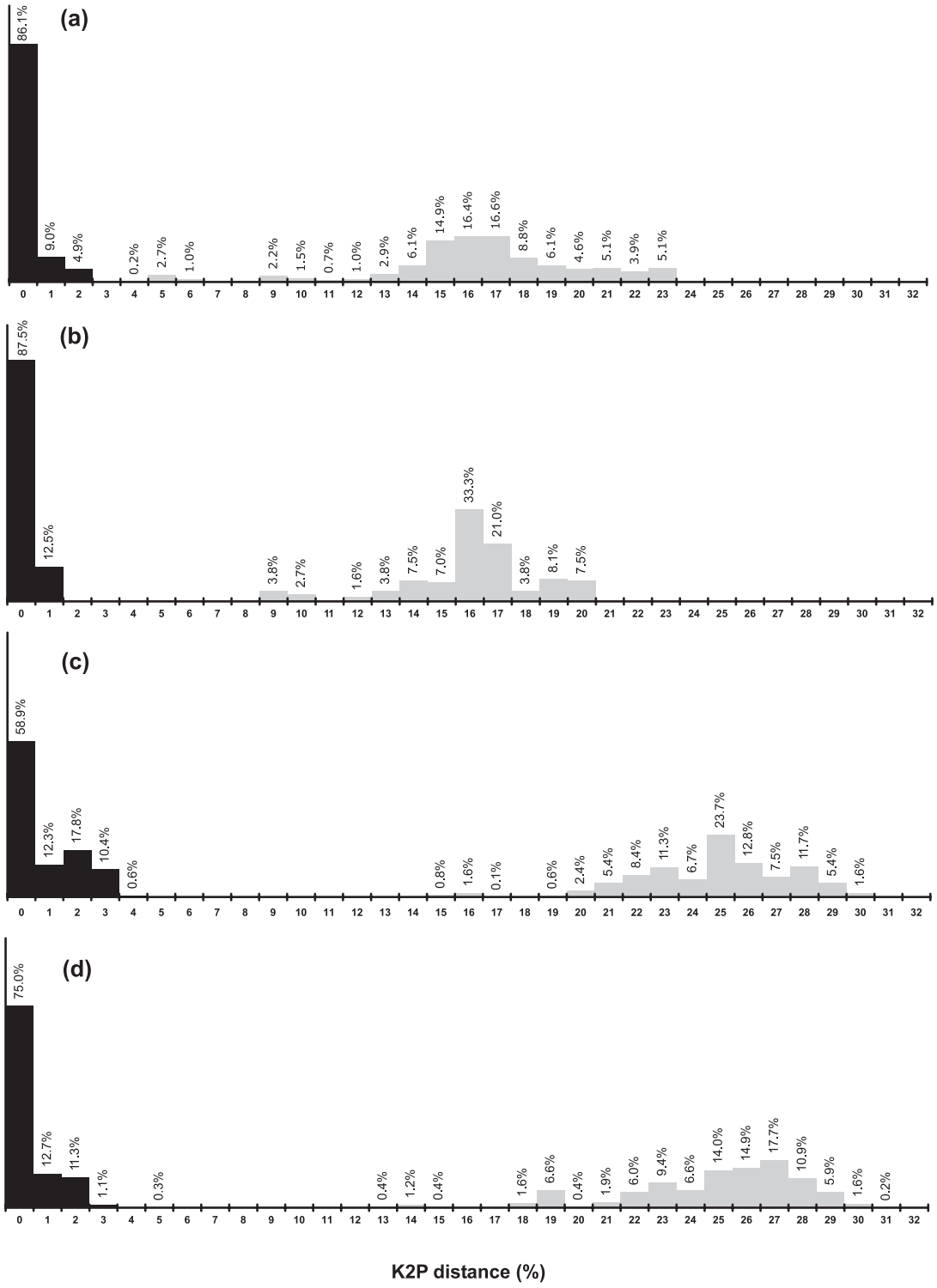
COI divergences among members of a species versus congeners

For members of the order Decapoda, the average K2P distance within species averaged 0.46%, while congeneric divergences averaged 17.16%, but ranged from a low 4.92% in the crab genus *Chionoecetes* to a high of 31.39% in the amphipod genus *Gammarus* (Table 1). Within-species divergences were below 1% for 86% of the within-species comparisons in decapods. The equivalent value in *Gammarus* was only 75%, while in *Daphnia* it was 59%. Although the *Daphnia* data set showed higher within-species distances, the congeneric distances were are also high, averaging 25.28%, so even in this genus there was a clear separation of conspecific and congeneric distances (Fig. 3). The distribution of K2P distances within different taxonomic categories (species, genus) for the Decapoda and as well for the genera *Pandalus*, *Daphnia*, and *Gammarus* is shown (Fig. 3).

Variation of GC content in crustacean COI genes

The availability of 25 complete mitochondrial sequences enabled examination of the relationship between GC content of the COI barcode region and that in the whole mitochondrial genome. This analysis revealed that the GC content of the barcode region was a very strong predictor ($r^2 = 0.81$, $p < 0.001$) of genomic shifts in nucleotide usage, motivating an examination of GC content in all crustaceans where 5′-COI information was available. The GC content of the

Fig. 3. Frequency distribution of conspecific (solid bars) and congeneric (shaded bars) mitochondrial cytochrome *c* oxidase I (COI) barcode distances (K2P) from pairwise comparisons among members of the order Decapoda and selected crustacean genera: (*a*) order Decapoda, (*b*) genus *Pandalus*, (*c*) genus *Daphnia*, and (*d*) genus *Gammarus*. Values below 0.1% are not displayed.



barcode region varied among the 617 species of crustaceans from 29.9% to 49.6% (Table 2). The highest GC content was observed in *Parartemia longicaudata* from saline lakes in Australia, while the lowest GC content was found in the amphipod *Hyperia galba*, a parasite of jellyfish. Members of the orders Amphipoda and Isopoda possessed the highest and lowest average GC content, respectively (41.2% vs. 38.7%). In all cases, the GC content decreased from the first

Table 2. Variation in GC content in the mitochondrial cytochrome *c* oxidase I (COI) barcode region among crustacean taxa.

				Codon position		
Taxon[a]	Min.	Mean	Max.	1st	2nd	3rd
Subphylum Crustacea (617)	29.86	38.98±0.14	49.62	49.35±0.14	43.05±0.06	24.21±0.33
Order Amphipoda (48)	35.02	40.39±0.53	49.39	47.54±0.42	43.61±0.17	29.82±1.34
Order Anostraca (35)	35.81	39.46±0.53	49.62	51.07±0.35	44.27±0.19	23.03±1.38
Subclass Copepoda (61)	33.50	38.69±0.38	44.73	48.34±0.28	42.02±0.12	25.71±0.97
Order Decapoda (138)	32.62	39.29±0.27	47.04	50.82±0.25	42.96±0.10	23.92±0.64
Order Isopoda (36)	33.17	38.93±0.54	45.69	47.95±0.55	44.00±0.33	24.83±1.23
Subclass Phyllopoda (55)	34.48	40.10±0.36	46.35	51.42±0.33	43.93±0.08	24.81±0.89

[a]Number of species analyzed are shown in parentheses.

to the third codon position, ranging from 48% to 52% in the former, but only from 23% to 31.6% in the latter. Variation in GC content was consistently higher in the third codon base, while the second base showed the least variation.

Discussion

This study establishes that the barcode region of COI has considerable potential as the foundation for a DNA barcoding identification system for crustaceans. A COI profile based on single representatives of 150 crustacean families proved 95% effective in placing newly encountered species to the right order. This high success makes it clear that a profile based on even a few thousand species will provide a highly effective tool for taxonomic assignments at this level. Further parameterization of the ordinal profile should begin by adding sequence records for each of the 849 crustacean families. Higher sampling intensity should be directed to families with accelerated rates of molecular evolution, and the present results provide some directions in this regard. Parasitic lineages often show rate acceleration (Hassanin 2006; J.R. deWaard and P.D.N. Hebert, unpublished data), and this pattern was apparent in the current study, as evidenced by the isolated positions of parasitic isopods in the NJ tree. As a result, particular sampling intensity should be directed to crustacean lineages with parasitic lifestyles to subdivide the misleading long branches and to create an effective system for placements deep in the taxonomic hierarchy.

The present study suggests that a COI-based system will regularly deliver species-level resolution for crustacean lineages. In fact, levels of sequence divergence among congeneric species of crustaceans averaged 17.16%, the highest value yet reported for any animal group. By comparison, congeneric species of lepidopterans show just 6.1% variation (Hebert et al. 2003), birds show 7.93% variation (Hebert et al. 2004), and fishes possess 9.93% divergence (Ward et al. 2005). Of course, the actual level of divergence among congeners is less critical than the ratio of the genetic divergence between species to that within species. Levels of intraspecific variation in crustaceans averaged 0.46%, values slightly higher than those reported in other groups (most range from 0.25% to 0.30%), but the interspecific to intraspecific ratios of sequence divergence were very large, ranging from 19.2 to 48.3. As a consequence, species recognition was straightforward in most cases (~95%). Based on these observations, we conclude that a COI barcoding system will deliver levels of species resolution comparable with those seen in tropical lepidopterans (98%, Hajibabaei et al. 2006), marine gastropods (96%, Meyer and Paulay 2005), fishes (100%, Ward et al. 2005), and birds (95%, Hebert et al. 2004).

We emphasize the need for a critical assessment of both barcode results and current taxonomic systems in cases of discordance. For example, the lack of barcode divergence between members of the mitten crab genus *Eriocheir* reflects a case where there is a growing consensus that some recognized species do not merit this status (discussed in detail in Chu et al. 2003 and Tang et al. 2003). In other cases, levels of barcode variation within species have surely been inflated by a failure of current taxonomic systems to recognize valid species. For example, the presence of two genetically divergent groups in the hermit crabs *Pagurus longicarpus* and *Pagurus pollicaris* is thought to reflect overlooked species (Young et al. 2002). Similarly, the freshwater shrimp *Paratya australiensis* includes several highly divergent mitochondrial lineages that are now thought to represent species that diversified in the Pliocene (Baker et al. 2004). Finally, both morphological and biogeographic data suggest the possibility of overlooked species within *Cherax tenuimanus* and *Cherax preissi* (Munasinghe et al. 2003).

No single approach can provide a definitive conclusion on species boundaries (but see Lee 2003). We emphasize that DNA barcoding is not a substitute for conventional taxonomic approaches. It seeks instead to flag cases of deep genetic divergence among individuals grouped as a single species that may indicate overlooked species. For example, we noted particularly high intraspecific divergences (3.1%) in *Gammarus oceanicus*, but individuals from the Baltic Sea, Iceland, and Hudson Bay showed an average within-species K2P divergence of only 0.43%. The extreme divergence values all reflected samples of this species from the St. Lawrence estuary, perhaps reflecting an overlooked species endemic to this region. In contrast with these cases, there are other cases where species recognized through past taxonomic work show little or no barcode divergence. Some of these cases may represent instances where current taxonomic systems inappropriately recognize variation as reflecting species status. However, other cases may reflect very young species. We emphasize that the recognition of taxo-

nomic boundaries in such cases is always demanding, often subjective, and best pursued through a weight of evidence approach that employs molecular, morphological, and ecological traits to reach a decision.

As the creation of a comprehensive COI barcode database for crustaceans will be a substantial undertaking, we stress that benefits will be diverse. COI can, for example, serve as a sentinel gene enabling the detection of lineages with unusual patterns of nucleotide usage or exceptional rates of evolution. The analysis of GC content in the present study demonstrates that the COI barcode region can be a predictor of the nucleotide usage of the entire mitochondrial genome. Similarly, the molecular rate acceleration of parasitic lineages revealed previously using other gene regions (Hassanin 2006; J.R. deWaard and P.D.N. Hebert, unpublished data) was evident in the current data set. As such, it will aid multigene systematics by allowing such studies to target taxa showing unusual attributes of nucleotide usage or evolutionary rates. The benefits of a barcode system will also extend into ecological work. For example, an estimated one-third of marine arthropods (thus mainly crustaceans) in 138 studies from the North Atlantic could not be identified to a species level (Schander and Willassen 2005). Schander and Willassen (2005) pointed out several relevant means by which improved ability to recognize marine species will benefit marine biological and ecological sciences in a number of ways (see also Böttger-Schnack et al. 2004). A particular gain could arise through species-level identification of larvae, enabling studies of larval dispersal and ecology, and benthic adult connectivity, all critical for management of crustacean fisheries (Tully et al. 2003). DNA barcoding will also allow the identification of crustacean prey items in stomach contents of fishes, birds, and other predators, aiding parameterization of food web models. For example, morphological approaches did not allow the species identification of several crustacean prey in the stomach contents of fulmars (*Fulmarus glacialis*) (Phillips et al. 1999). Other potential benefits of a COI barcode identification system for crustaceans include the identification of parasitic crustaceans at any developmental stage (e.g., Øines and Heuch 2005), as well as the detection of invasive crustacean species (Armstrong and Ball 2005). In summary, the present study reveals that a large-scale effort to assemble DNA barcode records for crustaceans will deliver a highly effective identification system with impacts on a broad range of research themes.

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References

Adamowicz, S.J., Hebert, P.D.N., and Marinone, M.C. 2004. Species diversity and endemism in the *Daphnia* of Argentina: a genetic investigation. Zool. J. Linn. Soc. **140**: 171–205.

Armstrong, K.F., and Ball, S.L. 2005. DNA barcodes for biosecurity: invasive species identification. Philos. Trans. R. Soc. Lond. B Biol. Sci. **360**: 1813–1823.

Baker, A.M., Hurwood, D.A., Krogh, M., and Hughes, J.M. 2004. Mitochondrial DNA signatures of restricted gene flow within divergent lineages of an atyid shrimp (*Paratya australiensis*). Heredity, **93**: 196–207.

Barrett, R.D.H., and Hebert, P.D.N. 2005. Identifying spiders through DNA barcodes. Can. J. Zool. **83**: 481–491.

Borror, D.J., Triplehorn, C.A., and Johnson, N.F. 1989. An introduction to the study of insects. 6th ed. Saunders College Publishing, Philadelphia, Pa.

Böttger-Schnack, R., Lenz, J., and Weikert, H. 2004. Are taxonomic details of relevance to ecologists? An example from oncaeid microcopepods of the Red Sea. Mar. Biol. **144**: 1127–1140.

Chu, K.H., Ho, H.Y., Li, C.P., and Chan, T.Y. 2003. Molecular phylogenetics of the mitten crab species in *Eriocheir*, *sensu lato* (Brachyura: Grapsidae). J. Crustac. Biol. **23**: 738–746.

Dasmahapatra, K.K., and Mallet, J. 2006. DNA barcodes: recent successes and future prospects. Heredity, **97**: 254–255.

Folmer, O., Black, M., Hoeh, W., Lutz, R., and Vrijenhoek, R. 1994. DNA primers for amplification of mitochondrial cytochrome *c* oxidase subunit I from diverse metazoan invertebrates. Mol. Mar. Biol. Biotechnol. **3**: 294–297.

Hajibabaei, M., deWaard, J.R., Ivanova, N.V., Ratnasingham, S., Dooh, R.T., Kirk, S.L., Mackie, P.M., and Hebert, P.D.N. 2005. Critical factors for assembling a high volume of DNA barcodes. Philos. Trans. R. Soc. Lond. B Biol. Sci. **360**: 1959–1967.

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**: 968–971.

Hassanin, A. 2006. Phylogeny of Arthropoda inferred from mitochondrial sequences: strategies for limiting the misleading effects of multiple changes in pattern and rates of substitution. Mol. Phylogenet. Evol. **38**: 100–116.

Hebert, P.D.N., Cywinska, A., Ball, S.L., and deWaard, J.R. 2003. Biological identifications through DNA barcodes. Proc. R. Soc. Lond. B Biol. Sci. **270**: 313–322.

Hebert, P.D.N., Stoeckle, M.Y., Zemlak, T.S., and Francis, C.M. 2004. Identification of birds through DNA barcodes. PLoS Biol. **2**: 1657–1663.

Janzen, D.H., Hajibabaei, M., Burns, J.M., Hallwachs, W., Remigio, E., and Hebert, P.D.N. 2005. Wedding biodiversity inventory of a large complex Lepidoptera fauna with DNA barcoding. Philos. Trans. R. Soc. Lond. B Biol. Sci. **360**: 1835–1845.

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

Kumar, S., Tamura, K., and Nei, M. 2004. MEGA3: integrated software for molecular evolutionary genetics analysis and sequence alignment. Brief. Bioinform. **5**: 150–163.

Lee, M.S.Y. 2003. Species concepts and species reality: salvaging a Linnaean rank. J. Evol. Biol. **16**: 179–188.

Macpherson, E., and Machordom, A. 2001. Phylogenetic relationships of species of *Raymunida* (Decapoda: Galatheidae) based on morphology and mitochondrial cytochrome oxidase sequences, with the recognition of four new species. J. Crustac. Biol. **21**: 696–714.

Martin, J.W., and Davis, G.E. 2001. An updated classification of the recent Crustacea. Natural History Museum, Los Angeles, Calif. Contrib. Sci. Ser. 39.

Meyer, C.P., and Paulay, G. 2005. DNA barcoding: error rates based on comprehensive sampling. PLoS Biol. **3**: 2229–2238.

Miller, A.D., Nguyen, T.T., Burridge, C.P., and Austin, C.M. 2004. Complete mitochondrial DNA sequence of the Australian freshwater crayfish, *Cherax destructor* (Crustacea: Decapoda: Parastacidae): a novel gene order revealed. Gene, **331**: 65–72.

Munasinghe, D.H.N., Murphy, N.P., and Austin, C.M. 2003. Utility of mitochondrial DNA sequences from four gene regions for systematic studies of Australian freshwater crayfish of the genus *Cherax* (Decapoda: Parastacidae). J. Crustac. Biol. **23**: 402–417.

Nei, M., and Kumar, S. 2000. Molecular evolution and phylogenetics. Oxford University Press, New York.

Nielsen, C. 1995. Animal evolution: interrelationships of the living phyla. Oxford University Press, Oxford, UK.

Øines, Ø., and Heuch, P.A. 2005. Identification of sea louse species of the genus *Caligus* using mtDNA. J. Mar. Biol. Assoc. U.K. **85**: 73–79.

Pfeiler, E., Hurtado, L.A., Knowles, L.L., Torre-Cosío, J., Bourillón-Moreno, L., Márquez-Farías, J.F., and Montemayor-López, G. 2005. Population genetics of the swimming crab *Callinectes bellicosus* (Brachyura: Portunidae) from the eastern Pacific Ocean. Mar. Biol. **146**: 559–569.

Phillips, R.A., Petersen, M.K., Lilliendahl, K., Solmundsson, J., Hamer, K.C., Camphuysen, C.J., and Zonfrillo, B. 1999. Diet of the northern fulmar *Fulmarus glacialis*: reliance on commercial fisheries? Mar. Biol. **135**: 159–170.

Ratnasingham, S., and Hebert, P.D.N. 2007. BOLD: The Barcode of Life Data System (www.barcodinglife.org). Mol. Ecol. Notes, doi:10.1111/j.1471-8286.2006.01678.x.

Roman, J., and Palumbi, S.R. 2004. A global invader at home: population structure of the green crab, *Carcinus maenas*, in Europe. Mol. Ecol. **13**: 2891–2898.

Schander, C., and Willassen, E. 2005. What can biological barcoding do for marine biology? Mar. Biol. Res. **1**: 79–83.

Schwenk, K., Sand, A., Boersma, M., Brehm, M., Mader, E., Offerhaus, D., and Spaak, P. 1998. Genetic markers, genealogies and biogeographic patterns in the Cladocera. Aquat. Ecol. **32**: 37–51.

Smith, M.A., Fisher, B., and Hebert, P.D.N. 2005. DNA barcoding for effective biodiversity assessment of a hyperdiverse arthropod group: the ants of Madagascar. Philos. Trans. R. Soc. Lond. B Biol. Sci. **360**: 1825–1834.

Tang, B., Zhou, K., Song, D., Yang, G., and Dai, A. 2003. Molecular systematics of the Asian mitten crabs, genus *Eriocheir* (Crustacea: Brachyura). Mol. Phylogenet. Evol. **29**: 309–316.

Thompson, J.D., Higgins, D.G., and Gibson, T.J. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res. **22**: 4673–4680.

Tully, O., Freire, J., and Addison, J. 2003. Crustacean fisheries. Fish. Res. **65**: 1–2.

Ward, R.D., Zemlak, T.S., Innes, B.H., Last, P.R., and Hebert, P.D.N. 2005. DNA barcoding Australia's fish species. Philos. Trans. R. Soc. Lond. B Biol. Sci. **360**: 1847–1957.

Young, A.M., Torres, C., Mack, J.E., and Cunningham, C.W. 2002. Morphological and genetic evidence for vicariance and refugium in Atlantic and Gulf of Mexico populations of the hermit crab *Pagurus longicarpus*. Mar. Biol. **140**: 1059–1066.

Appendix A

Table A1. List of specimens used in the profile of 150 crustacea families, sorted by family name.

GenBank accession No.	Class	Order	Family	Species	Length
AY145426	Maxillopoda	Calanoida	Acartiidae	*Acartia omorii*	624
EF432739	Malacostraca	Isopoda	Aegidae	*Rocinela angustata*	654
AF436034	Malacostraca	Decapoda	Aeglidae	*Aegla uruguayana*	657
AF531742	Maxillopoda	Calanoida	Aetideidae	*Gaetanus tenuispinus*	627
AF436036	Malacostraca	Decapoda	Albuneidae	*Blepharipoda occidentalis*	657
DQ889084	Malacostraca	Amphipoda	Ampeliscidae	*Byblis gaimardi*	623
DQ889076	Malacostraca	Anaspidacea	Anaspididae	*Anaspides tasmaniae*	657
DQ889126	Malacostraca	Amphipoda	Anisogammaridae	*Ramellogammarus* sp.	654
DQ889078	Malacostraca	Isopoda	Arcturidae	*Arcturus baffini*	644
DQ889096	Maxillopoda	Arguloida	Argulidae	*Dolops* sp.	624
DQ889079	Malacostraca	Isopoda	Armadillidiidae	*Armadillidium* sp.	657
NC_001620	Branchiopoda	Anostraca	Artemiidae	*Artemia franciscana*	624
DQ889085	Malacostraca	Isopoda	Asellidae	*Caecidotea* sp.	652
DQ889120	Malacostraca	Decapoda	Atyidae	*Paratya australiensis*	657
AF242660	Maxillopoda	Sessilia	Balanidae	*Semibalanus balanoides*	642
DQ889092	Malacostraca	Cumacea	Bodotriidae	*Cyclaspis caprella*	633
DQ889082	Malacostraca	Isopoda	Bopyridae	*Bopyroides hippolytes*	629
DQ889083	Branchiopoda	Cladocera	Bosminidae	*Bosmina* sp.	639
AF209064	Branchiopoda	Anostraca	Branchinectidae	*Branchinecta paludosa*	639
AF209059	Branchiopoda	Anostraca	Branchipodidae	*Parartemia contracta*	639
AF044057	Malacostraca	Decapoda	Bresiliidae	*Rimicaris exoculata*	605
DQ889086	Branchiopoda	Diplostraca	Caenestheriidae	*Caenestheriella setosa*	639
DQ889087	Maxillopoda	Calanoida	Calanidae	*Calanus glacialis*	624
AF436025	Malacostraca	Decapoda	Callianassidae	*Callichirus major*	649
DQ882037	Malacostraca	Decapoda	Calocarididae	*Calocaris investigatoris*	658

Table A1 (*continued*).

GenBank accession No.	Class	Order	Family	Species	Length
DQ889125	Malacostraca	Decapoda	Cambaridae	*Procambarus clarkii*	654
DQ882040	Malacostraca	Decapoda	Cancridae	*Cancer magister*	655
AY145434	Maxillopoda	Calanoida	Candaciidae	*Candacia ethiopica*	624
AF315015	Maxillopoda	Harpacticoida	Canuellidae	*Coullana* sp.	657
DQ889075	Malacostraca	Amphipoda	Caprellidae	*Aeginina longicornis*	644
AY428048	Maxillopoda	Sessilia	Catophragmidae	*Catomerus polymerus*	643
DQ356555	Maxillopoda	Calanoida	Centropagidae	*Boeckella gracilis*	636
DQ889088	Branchiopoda	Diplostraca	Cercopagididae	*Cercopagis pengoi*	639
AF209062	Branchiopoda	Anostraca	Chirocephalidae	*Artemiopsis stefanssoni*	639
DQ889089	Maxillopoda	Sessilia	Chthamalidae	*Chamaesipho* sp.	657
DQ889090	Branchiopoda	Cladocera	Chydoridae	*Chydorus* sp.	639
AF513651	Maxillopoda	Calanoida	Clausocalanidae	*Pseudocalanus mimus*	639
AF315009	Maxillopoda	Harpacticoida	Cletodidae	*Cletocamptus deitersi*	657
AY327387	Maxillopoda	Harpacticoida	Cletopsyllidae	*Bathycletopsyllus* sp.	603
AF205247	Malacostraca	Stomatopoda	Coronididae	*Parvisquilla multituberculata*	648
AY174366	Maxillopoda	Sessilia	Coronulidae	*Chelonibia testudinaria*	586
DQ889128	Malacostraca	Decapoda	Crangonidae	*Sclerocrangon boreas*	657
DQ889090	Malacostraca	Amphipoda	Crangonyctidae	*Crangonyx* sp.	637
DQ889093	Branchiopoda	Diplostraca	Cyclestheriidae	*Cyclestheria hislopi*	639
AF255791	Malacostraca	Isopoda	Cymothoidae	*Olencira praegustator*	583
DQ889094	Ostracoda	Podocopida	Cyprididae	*Cypridopsis vidua*	624
NC_000844	Branchiopoda	Diplostraca	Daphniidae	*Daphnia pulex*	624
AJ534411	Ostracoda	Podocopida	Darwinulidae	*Vestalenula molopoensis*	534
DQ889095	Maxillopoda	Calanoida	Diaptomidae	*Diaptomus* sp.	608
AF520442	Malacostraca	Cumacea	Diastylidae	*Diastylopsis thilenuisi*	657
DQ882097	Malacostraca	Decapoda	Diogenidae	*Paguristes turgidus*	658
DQ889097	Malacostraca	Decapoda	Dromiidae	—	644
DQ889118	Malacostraca	Amphipoda	Epimeriidae	*Paramphithoe hystrix*	644
AB091772	Maxillopoda	Calanoida	Eucalanidae	*Eucalanus bungii*	900
AF531749	Maxillopoda	Calanoida	Euchaetidae	*Paraeuchaeta biloba*	618
DQ889098	Malacostraca	Euphausiacea	Euphausiidae	*Euphausia superba*	633
DQ889127	Malacostraca	Amphipoda	Eusiridae	*Rhachotropis aculeata*	641
DQ882090	Malacostraca	Decapoda	Galatheidae	*Munida quadrispina*	658
DQ889100	Malacostraca	Amphipoda	Gammaridae	*Gammarus lacustris*	654
DQ889101	Malacostraca	Decapoda	Gnathophyllidae	*Gnathophyllum americanus*	657
AF048822	Malacostraca	Stomatopoda	Gonodactylidae	*Gonodactylus graphurus*	645
AF317338	Malacostraca	Decapoda	Grapsidae	*Grapsus albolineatus*	573
AF520447	Malacostraca	Cumacea	Gynodiastylidae	*Gynodiastylis* sp.	657
L43049	Maxillopoda	Harpacticoida	Harpactidae	*Tigriopus californicus*	606
AF205252	Malacostraca	Stomatopoda	Hemisquillidae	*Hemisquilla ensigera*	645
AF531745	Maxillopoda	Calanoida	Heterorhabdidae	*Heterorhabdus farrani*	642
AF048823	Malacostraca	Stomatopoda	Heterosquillidae	*Heterosquilla tricarinata*	655
DQ882084	Malacostraca	Decapoda	Hippolytidae	*Lebbeus groenlandicus*	505
DQ889102	Branchiopoda	Cladocera	Holopedidae	*Holopedium gibberum*	639
AF370852	Cephalocarida	Brachypoda	Hutchinsoniellidae	*Hutchinsoniella macracantha*	634
AY152752	Malacostraca	Amphipoda	Hyalellidae	*Hyalella* sp.	637
AY639937	Malacostraca	Amphipoda	Hyalidae	*Parhyale hawaiiensis*	900
DQ889133	Malacostraca	Amphipoda	Hyperiidae	*Themisto gaudichaudii*	657
DQ889111	Malacostraca	Isopoda	Idoteidae	*Mesidotea entomon*	657
AF451354	Malacostraca	Amphipoda	Iphimediidae	*Gnathiphimedia sexdentata*	624
DQ889106	Malacostraca	Amphipoda	Ischyroceridae	*Ischyrocerus latipes*	643
AF352297	Malacostraca	Cumacea	Lampropidae	*Lampropis quadriplicata*	626
AF436030	Malacostraca	Decapoda	Laomediidae	*Jaxea nocturna*	593
DQ889131	Malacostraca	Mysidacea	Lepidomysidae	*Tasmanomysis oculata*	633
DQ889107	Branchiopoda	Cladocera	Leptodoridae	*Leptodora kindtii*	638
AF137516	Malacostraca	Cumacea	Leuconidae	*Eudorella pusilla*	657
DQ889130	Maxillopoda	Cyclopoida	Lichomolgidae	*Stellicola* sp.	654

Table A1 (*continued*).

GenBank accession No.	Class	Order	Family	Species	Length
AF255780	Malacostraca	Isopoda	Ligiidae	*Ligia occidentalis*	583
AJ534412	Ostracoda	Podocopida	Limnocytheridae	*Limnocythere inopinata*	533
DQ882086	Malacostraca	Decapoda	Lithodidae	*Lithodes couesi*	639
DQ889115	Malacostraca	Lophogastrida	Lophogastridae	*Neognathophausia ingens*	630
DQ889108	Malacostraca	Decapoda	Luciferidae	*Lucifer* sp.	656
DQ889109	Branchiopoda	Diplostraca	Lynceidae	*Lynceus* sp.	639
DQ889077	Malacostraca	Amphipoda	Lysianassidae	*Anonyx nugax*	643
DQ889117	Branchiopoda	Cladocera	Macrothricidae	*Ophryoxus gracilis*	639
DQ889114	Malacostraca	Decapoda	Majidae	*Naxia* sp.	657
AF474106	Maxillopoda	Calanoida	Metridinidae	*Metridia lucens*	648
DQ889112	Branchiopoda	Cladocera	Moinidae	*Moina* sp.	639
DQ889119	Malacostraca	Mysidacea	Mysidae	*Paramysis intermedia*	618
AF520450	Malacostraca	Cumacea	Nannastacidae	*Campylaspis* sp.	657
AF125435	Malacostraca	Decapoda	Nematocarcinidae	*Nematocarcinus ensifer*	600
DQ889104	Malacostraca	Decapoda	Nephropidae	*Homarus americanus*	654
DQ889116	Ostracoda	Podocopida	Notodromadidae	*Notodromas monacha*	523
AF205234	Malacostraca	Stomatopoda	Odontodactylidae	*Odontodactylus scyllarus*	644
EF432738	Malacostraca	Amphipoda	Oedicerotidae	*Monoculodes borealis*	644
DQ882168	Malacostraca	Decapoda	Oplophoridae	*Systellaspis braueri*	654
DQ882047	Malacostraca	Decapoda	Oregoniidae	*Chionoecetes bairdi*	658
NC_003058	Malacostraca	Decapoda	Paguridae	*Pagurus longicarpus*	657
DQ889110	Malacostraca	Decapoda	Palaemonidae	*Macrobrachium* sp.	656
NC_004251	Malacostraca	Decapoda	Palinuridae	*Panulirus japonicus*	657
DQ882111	Malacostraca	Decapoda	Pandalidae	*Pandalus danae*	658
DQ882143	Malacostraca	Decapoda	Panopeidae	*Rhithropanopeus harrisii*	658
AF474110	Maxillopoda	Calanoida	Paracalanidae	*Paracalanus parvus*	648
AF493624	Malacostraca	Decapoda	Parastacidae	*Cherax crassimanus*	600
DQ882133	Malacostraca	Decapoda	Pasiphaeidae	*Pasiphaea pacifica*	658
AF217843	Malacostraca	Decapoda	Penaeidae	*Penaeus monodon*	657
AF255775	Malacostraca	Isopoda	Phreatoicidae	*Colubotelson thomsoni*	583
AF317341	Malacostraca	Decapoda	Plagusiidae	*Plagusia immaculata*	573
DQ889099	Branchiopoda	Cladocera	Podonidae	*Evadne spinifera*	639
AF209063	Branchiopoda	Anostraca	Polyartemiidae	*Polyartemiella hazeni*	639
DQ889121	Branchiopoda	Cladocera	Polyphemidae	*Polyphemus pediculus*	639
AY145429	Maxillopoda	Calanoida	Pontellidae	*Calanopia thompsoni*	624
AY189478	Malacostraca	Amphipoda	Pontogammaridae	*Obesogammarus crassus*	654
DQ889122	Malacostraca	Amphipoda	Pontoporeiidae	*Pontoporeia femorata*	653
DQ889123	Malacostraca	Isopoda	Porcellionidae	*Porcellio spinicornis*	654
DQ889124	Malacostraca	Decapoda	Portunidae	*Portunus pelagicus*	657
AF399980	Malacostraca	Decapoda	Potamidae	*Potamon fluviatilis*	539
AF205253	Malacostraca	Stomatopoda	Protosquillidae	*Chorisquilla excavata*	644
AF137514	Malacostraca	Cumacea	Pseudocumatidae	*Pseudocuma similis*	657
AY145436	Maxillopoda	Calanoida	Pseudodiaptomidae	*Pseudodiaptomus marinus*	624
AF205245	Malacostraca	Stomatopoda	Pseudosquillidae	*Pseudosquilla ciliata*	646
AF346400	Malacostraca	Decapoda	Raninidae	*Ranina ranina*	632
AY456188	Maxillopoda	Pedunculata	Scalpellidae	*Pollicipes polymerus*	658
DQ889105	Malacostraca	Decapoda	Scyllaridae	*Ibacus peronii*	654
DQ882149	Malacostraca	Decapoda	Sergestidae	*Sergestes similis*	658
DQ889129	Branchiopoda	Diplostraca	Sididae	*Sida crystallina*	639
AF370851	Remipedia	Nectiopoda	Speleonectidae	*Speleonectes gironensis*	613
AF255785	Malacostraca	Isopoda	Sphaeromatidae	*Sphaeroma quadridentatum*	544
AF531751	Maxillopoda	Calanoida	Spinocalanidae	*Spinocalanus abyssalis*	507
NC_006081	Malacostraca	Stomatopoda	Squillidae	*Squilla mantis*	658
AF125441	Malacostraca	Decapoda	Stenopodidae	*Stenopus hispidus*	600
AF531752	Maxillopoda	Calanoida	Stephidae	*Stephos longipes*	621
AF209065	Branchiopoda	Anostraca	Streptocephalidae	*Streptocephalus dorothae*	639
DQ889103	Malacostraca	Decapoda	Sundathelphusidae	*Holthuisana transversa*	653

Table A1 (*concluded*).

GenBank accession No.	Class	Order	Family	Species	Length
AF205257	Malacostraca	Stomatopoda	Takuidae	*Taku spinosocarinatus*	647
AY152751	Malacostraca	Amphipoda	Talitridae	*Orchestia uhleri*	637
DQ889132	Maxillopoda	Calanoida	Temoridae	*Temora stylifera*	636
AB126701	Maxillopoda	Sessilia	Tetraclitidae	*Tetraclita japonica*	658
AF209066	Branchiopoda	Anostraca	Thamnocephalidae	*Thamnocephalus platyurus*	639
AF474112	Maxillopoda	Calanoida	Tortanidae	*Tortanus derjugini*	617
DQ889134	Malacostraca	Decapoda	Trapeziidae	*Trapezia rufopunctata*	644
DQ889135	Branchiopoda	Notostraca	Triopsidae	*Triops australiensis*	639
DQ882062	Malacostraca	Decapoda	Varunidae	*Eriocheir sinensis*	623
DQ889081	Malacostraca	Decapoda	Xanthidae	*Atergatis floridus*	656
DQ889113	Ostracoda	Myodocopida	—	—	655

Table A2. List of 100 specimens used as test taxa in the amino acid profile of the crustacea.

GenBank accession No.	Class	Order	Family	Species	Length
DQ889136	Branchiopoda	Cladocera	Macrothricidae	*Acantholeberis curvirostris*	639
DQ889137	Branchiopoda	Cladocera	Chydoridae	*Acroperus harpae*	639
DQ889138	Branchiopoda	Cladocera	Chydoridae	*Alona setulosa*	639
DQ889139	Branchiopoda	Cladocera	Chydoridae	*Alonella exigua*	639
DQ882032	Malacostraca	Decapoda	Crangonidae	*Argis lar*	658
AY456187	Maxillopoda	Arguloida	Argulidae	*Argulus americanus*	658
AY531772	Malacostraca	Isopoda	Asellidae	*Asellus aquaticus*	651
AF308940	Branchiopoda	Anostraca	Thamnocephalidae	*Branchinella pinnata*	639
DQ889140	Branchiopoda	Diplostraca	Cercopagididae	*Bythotrephes* sp.	639
AF332791	Maxillopoda	Calanoida	Calanidae	*Calanoides acutus*	639
AY428047	Maxillopoda	Pedunculata	Scalpellidae	*Calantica spinosa*	677
DQ889141	Malacostraca	Amphipoda	Caprellidae	*Caprella unica*	641
AY616445	Malacostraca	Decapoda	Portunidae	*Carcinus aestuarii*	498
DQ889142	Maxillopoda	Calanoida	Centropagidae	*Centropages furcatus*	636
AY188999	Branchiopoda	Anostraca	Chirocephalidae	*Chirocephalus ruffoi*	654
DQ882053	Malacostraca	Decapoda	Majidae	*Chorilia longipes*	655
AF234819	Maxillopoda	Sessilia	Chthamalidae	*Chthamalus anisopoma*	656
AF462313	Maxillopoda	Calanoida	Clausocalanidae	*Clausocalanus minor*	648
AF520446	Malacostraca	Cumacea	Diastylidae	*Colurostylis longicaudata*	658
DQ882057	Malacostraca	Decapoda	Crangonidae	*Crangon alaskensis*	658
AF255776	Malacostraca	Isopoda	Phreatoicidae	*Crenoicus buntiae*	580
AF332789	Maxillopoda	Calanoida	Calanidae	*Ctenocalanus citer*	639
AF520449	Malacostraca	Cumacea	Nannastacidae	*Cumella* sp.	658
DQ889143	Branchiopoda	Diplostraca	Sididae	*Diaphanosoma* sp.	639
AF137510	Malacostraca	Cumacea	Diastylidae	*Diastylis sculpta*	658
DQ889144	Malacostraca	Amphipoda	Pontoporeiidae	*Diporeia hoyi*	649
DQ889145	Maxillopoda	Calanoida	Clausocalanidae	*Drepanopus* sp.	621
DQ889146	Branchiopoda	Cladocera	Macrothricidae	*Drepanothrix dentata*	639
AF451352	Malacostraca	Amphipoda	Iphimediidae	*Echiniphimedia echinata*	624
DQ889147	Malacostraca	Amphipoda	Gammaridae	*Echinogammarus ischnus*	640
AF493633	Malacostraca	Decapoda	Parastacidae	*Engaeus strictifrons*	600
AF520445	Malacostraca	Cumacea	Bodotriidae	*Eocuma longicorne*	657
AF451342	Malacostraca	Amphipoda	Epimeriidae	*Epimeria reoproi*	644
DQ889148	Malacostraca	Amphipoda	Ischyroceridae	*Ericthonius* sp.	644
DQ882073	Malacostraca	Decapoda	Hippolytidae	*Eualus biunguis*	624
AF209061	Branchiopoda	Anostraca	Chirocephalidae	*Eubranchipus* sp.	639
AF234820	Maxillopoda	Sessilia	Chthamalidae	*Euraphia eastropacensis*	598
DQ889149	Branchiopoda	Cladocera	Chydoridae	*Eurycercus longirostris*	639
AY145427	Maxillopoda	Calanoida	Temoridae	*Eurytemora pacifica*	624

Table A2 (*continued*).

GenBank accession No.	Class	Order	Family	Species	Length
DQ889150	Malacostraca	Amphipoda	Eusiridae	*Eusirus cuspidatus*	644
DQ889151	Malacostraca	Isopoda	Sphaeromatidae	*Exosphaeroma* sp.	654
AF395115	Malacostraca	Decapoda	Penaeidae	*Fenneropenaeus* sp.	657
AF255781	Malacostraca	Isopoda	Idoteidae	*Glyptidotea lichtensteini*	574
AF205231	Malacostraca	Stomatopoda	Gonodactylidae	*Gonodactylellus hendersoni*	648
AF205224	Malacostraca	Stomatopoda	Gonodactylidae	*Gonodactylinus viridis*	627
AF205242	Malacostraca	Stomatopoda	Gonodactylidae	*Gonodactylopsis komodoensis*	645
DQ889152	Branchiopoda	Cladocera	Chydoridae	*Graptoleberis testudinaria*	639
AF205239	Malacostraca	Stomatopoda	Protosquillidae	*Haptosquilla glyptocercus*	646
AF317340	Malacostraca	Decapoda	Varunidae	*Hemigrapsus sanguineus*	573
AF061781	Malacostraca	Cumacea	Lampropidae	*Hemilamprops californicus*	658
DQ882079	Malacostraca	Decapoda	Oregoniidae	*Hyas lyratus*	658
DQ889153	Malacostraca	Amphipoda	Hyperiidae	*Hyperia galba*	643
DQ889154	Malacostraca	Amphipoda	Lysianassidae	*Ichnopus spinicornis*	626
DQ889155	Malacostraca	Isopoda	Idoteidae	*Idotea granulosa*	636
AF451347	Malacostraca	Amphipoda	Iphimediidae	*Iphimediella rigida*	618
AF339473	Malacostraca	Decapoda	Palinuridae	*Jasus edwardsii*	639
AY145428	Maxillopoda	Calanoida	Pontellidae	*Labidocera rotunda*	624
DQ889156	Branchiopoda	Notostraca	Triopsidae	*Lepidurus* sp.	639
AF520444	Malacostraca	Cumacea	Bodotriidae	*Leptocuma* sp.	633
DQ889157	Branchiopoda	Diplostraca	Limnadiidae	*Limnadia* sp.	639
DQ889158	Maxillopoda	Calanoida	Centropagidae	*Limnocalanus macrurus*	631
DQ889159	Malacostraca	Mysidacea	Mysidae	*Limnomysis benedeni*	634
AF260843	Malacostraca	Isopoda	Cymothoidae	*Lironeca vulgaris*	617
DQ882088	Malacostraca	Decapoda	Lithodidae	*Lopholithodes foraminatus*	658
DQ889160	Branchiopoda	Cladocera	Macrothricidae	*Macrothrix* sp.	639
AF137520	Malacostraca	Cumacea	Bodotriidae	*Mancocuma stellifera*	675
AJ319742	Ostracoda	Podocopida	Cyprididae	*Mesocyprideis irsacae*	462
DQ889161	Malacostraca	Mysidacea	Mysidae	*Mysis americana*	654
DQ889162	Ostracoda	Podocopida	Cyprididae	*Mytilocypris ambiguosa*	656
AF332793	Maxillopoda	Calanoida	Calanidae	*Nannocalanus minor*	613
AF513650	Maxillopoda	Calanoida	Calanidae	*Neocalanus robustior*	639
AF205235	Malacostraca	Stomatopoda	Gonodactylidae	*Neogonodactylus bredini*	648
DQ882094	Malacostraca	Decapoda	Oplophoridae	*Notostomus japonicus*	647
DQ889163	Malacostraca	Euphausiacea	Euphausiidae	*Nyctiphanes australis*	633
AY428049	Maxillopoda	Sessilia	Chthamalidae	*Octomeris angulosa*	639
DQ889164	Malacostraca	Amphipoda	Lysianassidae	*Onisimus glacialis*	639
DQ889165	Malacostraca	Decapoda	Cambaridae	*Orconectes propinquus*	653
DQ889166	Maxillopoda	Calanoida	Centropagidae	*Osphranticum labronectum*	636
AF137512	Malacostraca	Cumacea	Diastylidae	*Oxyurostylis smithi*	658
DQ882103	Malacostraca	Decapoda	Palaemonidae	*Palaemon elegans*	658
DQ882106	Malacostraca	Decapoda	Pandalidae	*Pandalopsis dispar*	657
DQ356574	Maxillopoda	Calanoida	Centropagidae	*Parabroteas sarsia*	636
AF052393	Malacostraca	Mysidacea	Mysidae	*Paramesopodopsis rufa*	651
AF255783	Malacostraca	Isopoda	Idoteidae	*Paridotea ungulata*	564
DQ889167	Branchiopoda	Cladocera	Podonidae	*Pleopis* sp.	639
DQ889168	Branchiopoda	Cladocera	Chydoridae	*Pleuroxus denticulatus*	639
DQ889169	Branchiopoda	Cladocera	Podonidae	*Podon leuckarti*	639
AF352302	Malacostraca	Cumacea	Bodotriidae	*Pomacuma australiae*	675
DQ889170	Malacostraca	Amphipoda	Gammaridae	*Pontogammarus maeoticus*	657
AF283874	Malacostraca	Decapoda	Galatheidae	*Raymunida dextralis*	657
AF531746	Maxillopoda	Calanoida	Eucalanidae	*Rhincalanus gigas*	633
DQ889171	Branchiopoda	Diplostraca	Daphniidae	*Scapholeberis rammneri*	639
DQ889172	Branchiopoda	Diplostraca	Daphniidae	*Simocephalus vetulus*	639
AF260846	Malacostraca	Isopoda	Sphaeromatidae	*Sphaeramene polytylotus*	579
DQ882157	Malacostraca	Decapoda	Hippolytidae	*Spirontocaris lamellicornis*	658
AF052394	Malacostraca	Mysidacea	Mysidae	*Tenagomysis australis*	645

Table A2 (*concluded*).

GenBank accession No.	Class	Order	Family	Species	Length
AY430813	Maxillopoda	Sessilia	Chthamalidae	*Tetrachthamalus oblitteratus*	603
DQ889173	Malacostraca	Decapoda	Scyllaridae	*Thenus orientalis*	657
AF352301	Malacostraca	Cumacea	Bodotriidae	*Vaunthompsonia cristata*	658
DQ889174	Malacostraca	Amphipoda	Gammaridae	*Weiprechtia pinguis*	644

Table A3. List of decapod specimens for which COI barcodes were acquired in this study, together with their country of collection, type of archive at the Biodiversity Institute of Ontario, primer pair used, and sequence length.

GenBank accession No.	Process ID	Species	Family	Country	Archive type	Primer pair[a]	Length
DQ882026	FCDPA135-04	*Acantholithodes hispidus*	Lithodidae	Canada	Specimen	FolA–FolB	658
DQ882029	FCDPA020-04	*Argis alaskensis*	Crangonidae	Canada	Specimen	FolA–FolB	658
DQ882028	FCDPA021-04	*Argis alaskensis*	Crangonidae	Canada	Specimen	FolA–FolB	654
DQ882027	FCDPA106-04	*Argis alaskensis*	Crangonidae	Canada	Specimen	FolA–FolB	658
DQ882032	FCDPA022-04	*Argis lar*	Crangonidae	Canada	Specimen	CrustF1–FolB	658
DQ882031	FCDPA023-04	*Argis lar*	Crangonidae	Canada	Specimen	CrustF1–FolB	658
DQ882030	FCDPA107-04	*Argis lar*	Crangonidae	Canada	Specimen	FolA–FolB	650
DQ882033	FCDPA050-04	*Bentheogennema borealis*	Benthesicymidae	Canada	Tissue	FolA–FolB	618
DQ882034	FCDPA051-04	*Bentheogennema borealis*	Benthesicymidae	Canada	Tissue	FolA–FolB	616
DQ882035	FCDPA111-04	*Calocarides spinulicauda*	Axiidae	Canada	Specimen	FolA–FolB	638
DQ882037	FCDPA052-04	*Calocaris investigatoris*	Calocarididae	Canada	Tissue	FolA–FolB	658
DQ882036	FCDPA053-04	*Calocaris investigatoris*	Calocarididae	Canada	Tissue	FolA–FolB	614
DQ882040	FCDPA061-04	*Cancer magister*	Cancridae	Canada	Tissue	CrustF1–FolB	655
DQ882038	FCDPA150-04	*Cancer magister*	Cancridae	Canada	Tissue	FolA–FolB	624
DQ882039	FCDPA151-04	*Cancer magister*	Cancridae	Canada	Tissue	FolA–FolB	638
DQ882041	FCDPA062-04	*Cancer oregonensis*	Cancridae	Canada	Tissue	CrustF1–FolB	616
DQ882042	FCDPA063-04	*Cancer oregonensis*	Cancridae	Canada	Tissue	CrustF1–FolB	511
DQ882044	FCDPA166-05	*Cancer productus*	Cancridae	Canada	Tissue	FolA–FolB	658
DQ882043	FCDPA064-04	*Cancer productus*	Cancridae	Canada	Tissue	FolA–FolB	605
DQ882045	FCDPA065-04	*Chionoecetes angulatus*	Oregoniidae	Canada	Tissue	FolA–FolB	620
DQ882046	FCDPA066-04	*Chionoecetes angulatus*	Oregoniidae	Canada	Tissue	FolA–FolB	520
DQ882049	FCDPA067-04	*Chionoecetes bairdi*	Oregoniidae	Canada	Tissue	FolA–FolB	652
DQ882048	FCDPA068-04	*Chionoecetes bairdi*	Oregoniidae	Canada	Tissue	CrustF1–FolB	643
DQ882047	FCDPA138-04	*Chionoecetes bairdi*	Oregoniidae	Canada	Specimen	FolA–FolB	658
DQ882051	FCDPA069-04	*Chionoecetes tanneri*	Oregoniidae	Canada	Tissue	FolA–FolB	523
DQ882050	FCDPA070-04	*Chionoecetes tanneri*	Oregoniidae	Canada	Tissue	FolA–FolB	656
DQ882054	FCDPA072-04	*Chorilia longipes*	Majidae	Canada	Specimen	CrustF1–FolB	552
DQ882053	FCDPA073-04	*Chorilia longipes*	Majidae	Canada	Tissue	FolA–FolB	655
DQ882052	FCDPA139-04	*Chorilia longipes*	Majidae	Canada	Specimen	FolA–FolB	656
DQ882055	FCDPA024-04	*Crangon abyssorum*	Crangonidae	Canada	Tissue	FolA–FolB	653
DQ882056	FCDPA025-04	*Crangon abyssorum*	Crangonidae	Canada	Tissue	FolA–FolB	645
DQ882057	FCDPA116-04	*Crangon alaskensis*	Crangonidae	Canada	Specimen	CrustF1–FolB	658
DQ882058	FCDPA117-04	*Crangon alaskensis*	Crangonidae	Canada	Specimen	FolA–FolB	655
DQ882060	FCDPA026-04	*Crangon communis*	Crangonidae	Canada	Specimen	CrustF1–FolB	574
DQ882061	FCDPA027-04	*Crangon communis*	Crangonidae	Canada	Specimen	CrustF1–FolB	658
DQ882059	FCDPA154-04	*Crangon communis*	Crangonidae	Canada	Specimen	CrustF1–FolB	630
DQ882062	FCDPA074-04	*Eriocheir sinensis*	Varunidae	Poland	Tissue	CrustF1–FolB	623
DQ882065	FCDPA123-04	*Eualus avinus*	Hippolytidae	Canada	Specimen	FolA–FolB	658
DQ882064	FCDPA124-04	*Eualus avinus*	Hippolytidae	Canada	Specimen	FolA–FolB	658
DQ882063	FCDPA125-04	*Eualus avinus*	Hippolytidae	Canada	Specimen	FolA–FolB	658
DQ882069	FCDPA034-04	*Eualus barbatus*	Hippolytidae	Canada	Specimen	FolA–FolB	651
DQ882070	FCDPA035-04	*Eualus barbatus*	Hippolytidae	Canada	Specimen	FolA–FolB	658

Table A3 (*continued*).

GenBank accession No.	Process ID	Species	Family	Country	Archive type	Primer pair[a]	Length
DQ882066	FCDPA109-04	*Eualus barbatus*	Hippolytidae	Canada	Specimen	FolA–FolB	645
DQ882067	FCDPA110-04	*Eualus barbatus*	Hippolytidae	Canada	Specimen	FolA–FolB	639
DQ882068	FCDPA140-04	*Eualus barbatus*	Hippolytidae	Canada	Specimen	FolA–FolB	643
DQ882074	FCDPA036-04	*Eualus biunguis*	Hippolytidae	Canada	Tissue	FolA–FolB	658
DQ882073	FCDPA037-04	*Eualus biunguis*	Hippolytidae	Canada	Tissue	FolA–FolB	624
DQ882072	FCDPA038-04	*Eualus biunguis*	Hippolytidae	Canada	Tissue	FolA–FolB	615
DQ882071	FCDPA039-04	*Eualus biunguis*	Hippolytidae	Canada	Tissue	FolA–FolB	624
DQ882075	FCDPA087-04	*Eualus gaimardi*	Hippolytidae	Canada	Specimen	FolA–FolB	659
DQ882078	FCDPA040-04	*Eualus suckleyi*	Hippolytidae	Canada	Tissue	FolA–FolB	624
DQ882076	FCDPA126-04	*Eualus suckleyi*	Hippolytidae	Canada	Specimen	FolA–FolB	656
DQ882077	FCDPA127-04	*Eualus suckleyi*	Hippolytidae	Canada	Specimen	FolA–FolB	657
DQ882079	FCDPA071-04	*Hyas lyratus*	Oregoniidae	Canada	Tissue	FolA–FolB	658
DQ882080	FCDPA130-04	*Hymenodora frontalis*	Oplophoridae	Canada	Tissue	FolA–FolB	658
DQ882081	FCDPA141-04	*Hymenodora frontalis*	Oplophoridae	Canada	Tissue	FolA–FolB	608
DQ882084	FCDPA081-04	*Lebbeus groenlandicus*	Hippolytidae	Canada	Specimen	FolA–FolB	639
DQ882083	FCDPA032-04	*Lebbeus groenlandicus*	Hippolytidae	Canada	Tissue	FolA–FolB	505
DQ882082	FCDPA033-04	*Lebbeus groenlandicus*	Hippolytidae	Canada	Tissue	FolA–FolB	614
DQ882086	FCDPA054-04	*Lithodes couesi*	Lithodidae	Canada	Tissue	FolA–FolB	639
DQ882085	FCDPA055-04	*Lithodes couesi*	Lithodidae	Canada	Tissue	FolA–FolB	619
DQ882089	FCDPA056-04	*Lopholithodes foraminatus*	Lithodidae	Canada	Tissue	CrustF1–FolB	638
DQ882088	FCDPA136-04	*Lopholithodes foraminatus*	Lithodidae	Canada	Tissue	FolA–FolB	658
DQ882087	FCDPA148-04	*Lopholithodes foraminatus*	Lithodidae	Canada	Tissue	CrustF1–FolB	642
DQ882090	FCDPA058-04	*Munida quadrispina*	Galatheidae	Canada	Tissue	FolA–FolB	658
DQ882092	FCDPA059-04	*Munida quadrispina*	Galatheidae	Canada	Specimen	FolA–FolB	658
DQ882091	FCDPA060-04	*Munida quadrispina*	Galatheidae	Canada	Tissue	FolA–FolB	603
DQ882093	FCDPA137-04	*Munidopsis quadrata*	Galatheidae	Canada	Tissue	FolA–FolB	658
DQ882094	FCDPA131-04	*Notostomus japonicus*	Oplophoridae	Canada	Tissue	FolA–FolB	647
DQ882095	FCDPA089-04	*Orconectes immunis*	Cambaridae	Canada	Specimen	FolA–FolB	632
DQ882096	FCDPA075-04	*Orconectes limosus*	Cambaridae	Canada	Tissue	CrustF1–FolB	632
DQ882097	FCDPA134-04	*Paguristes turgidus*	Diogenidae	Canada	Specimen	FolA–FolB	658
DQ882100	FCDPA145-04	*Paguristes turgidus*	Diogenidae	Canada	Specimen	FolA–FolB	614
DQ882099	FCDPA146-04	*Paguristes turgidus*	Diogenidae	Canada	Specimen	FolA–FolB	645
DQ882098	FCDPA147-04	*Paguristes turgidus*	Diogenidae	Canada	Specimen	FolA–FolB	643
DQ882101	FCDPA160-05	*Palaemon* sp.	Palaemonidae	Poland	Specimen	FolA–FolB	658
DQ882103	FCDPA157-05	*Palaemon elegans*	Palaemonidae	Poland	Specimen	FolA–FolB	658
DQ882104	FCDPA158-05	*Palaemon elegans*	Palaemonidae	Poland	Specimen	FolA–FolB	658
DQ882102	FCDPA159-05	*Palaemon elegans*	Palaemonidae	Poland	Specimen	FolA–FolB	658
DQ882105	FCDPA161-05	*Palaemon elegans*	Palaemonidae	Poland	Specimen	FolA–FolB	658
DQ882107	FCDPA018-04	*Pandalopsis dispar*	Pandalidae	Canada	Specimen	FolA–FolB	654
DQ882108	FCDPA019-04	*Pandalopsis dispar*	Pandalidae	Canada	Specimen	FolA–FolB	653
DQ882106	FCDPA105-04	*Pandalopsis dispar*	Pandalidae	Canada	Specimen	FolA–FolB	657
DQ882112	FCDPA001-04	*Pandalus danae*	Pandalidae	Canada	Tissue	FolA–FolB	626
DQ882111	FCDPA113-04	*Pandalus danae*	Pandalidae	Canada	Specimen	FolA–FolB	658

Table A3 (*continued*).

GenBank accession No.	Process ID	Species	Family	Country	Archive type	Primer pair[a]	Length
DQ882110	FCDPA114-04	*Pandalus danae*	Pandalidae	Canada	Specimen	FolA–FolB	654
DQ882109	FCDPA115-04	*Pandalus danae*	Pandalidae	Canada	Specimen	FolA–FolB	654
DQ882113	FCDPA002-04	*Pandalus goniurus*	Pandalidae	Canada	Tissue	FolA–FolB	614
DQ882116	FCDPA003-04	*Pandalus hypsinotus*	Pandalidae	Canada	Tissue	FolA–FolB	604
DQ882115	FCDPA004-04	*Pandalus hypsinotus*	Pandalidae	Canada	Tissue	FolA–FolB	656
DQ882114	FCDPA005-04	*Pandalus hypsinotus*	Pandalidae	Canada	Tissue	FolA–FolB	658
DQ882118	FCDPA006-04	*Pandalus jordani*	Pandalidae	Canada	Specimen	CrustF1–FolB	652
DQ882117	FCDPA007-04	*Pandalus jordani*	Pandalidae	Canada	Specimen	CrustF1–FolB	613
DQ882120	FCDPA008-04	*Pandalus jordani*	Pandalidae	Canada	Specimen	CrustF1–FolB	658
DQ882119	FCDPA009-04	*Pandalus jordani*	Pandalidae	Canada	Specimen	CrustF1–FolB	658
DQ882121	FCDPA010-04	*Pandalus mortagui tridens*	Pandalidae	Canada	Tissue	CrustF1–FolB	616
DQ882123	FCDPA011-04	*Pandalus mortagui tridens*	Pandalidae	Canada	Tissue	CrustF1–FolB	618
DQ882122	FCDPA012-04	*Pandalus mortagui tridens*	Pandalidae	Canada	Tissue	CrustF1–FolB	615
DQ882126	FCDPA013-04	*Pandalus platyceros*	Pandalidae	Canada	Specimen	CrustF1–FolB	538
DQ882125	FCDPA014-04	*Pandalus platyceros*	Pandalidae	Canada	Specimen	CrustF1–FolB	658
DQ882124	FCDPA153-04	*Pandalus platyceros*	Pandalidae	Canada	Specimen	CrustF1–FolB	643
DQ882129	FCDPA015-04	*Pandalus stenolepis*	Pandalidae	Canada	Specimen	FolA–FolB	602
DQ882128	FCDPA016-04	*Pandalus stenolepis*	Pandalidae	Canada	Specimen	CrustF2–FolB	653
DQ882127	FCDPA017-04	*Pandalus stenolepis*	Pandalidae	Canada	Specimen	FolA–FolB	617
DQ882130	FCDPA057-04	*Paralomis multispina*	Lithodidae	Canada	Tissue	FolA–FolB	615
DQ882133	FCDPA041-04	*Pasiphaea pacifica*	Pasiphaeidae	Canada	Specimen	CrustF1–FolB	658
DQ882134	FCDPA042-04	*Pasiphaea pacifica*	Pasiphaeidae	Canada	Specimen	CrustF1–FolB	648
DQ882135	FCDPA043-04	*Pasiphaea pacifica*	Pasiphaeidae	Canada	Specimen	CrustF1–FolB	657
DQ882132	FCDPA128-04	*Pasiphaea pacifica*	Pasiphaeidae	Canada	Specimen	FolA–FolB	659
DQ882131	FCDPA129-04	*Pasiphaea pacifica*	Pasiphaeidae	Canada	Specimen	FolA–FolB	658
DQ882137	FCDPA044-04	*Pasiphaea tarda*	Pasiphaeidae	Canada	Tissue	FolA–FolB	616
DQ882136	FCDPA045-04	*Pasiphaea tarda*	Pasiphaeidae	Canada	Tissue	CrustF2–FolB	656
DQ882139	FCDPA047-04	*Pasiphaea tarda*	Pasiphaeidae	Canada	Specimen	CrustF2–FolB	658
DQ882138	FCDPA048-04	*Pasiphaea tarda*	Pasiphaeidae	Canada	Specimen	CrustF2–FolB	641
DQ882143	FCDPA162-05	*Rhithropanopeus harrisii*	Panopeidae	Poland	Specimen	FolA–FolB	658
DQ882142	FCDPA163-05	*Rhithropanopeus harrisii*	Panopeidae	Poland	Specimen	FolA–FolB	658
DQ882140	FCDPA164-05	*Rhithropanopeus harrisii*	Panopeidae	Poland	Specimen	FolA–FolB	658
DQ882141	FCDPA165-05	*Rhithropanopeus harrisii*	Panopeidae	Poland	Specimen	FolA–FolB	658
DQ882144	FCDPA079-04	*Sclerocrangon boreas*	Crangonidae	Canada	Specimen	FolA–FolB	659
DQ882145	FCDPA080-04	*Sclerocrangon boreas*	Crangonidae	Canada	Specimen	FolA–FolB	659
DQ882146	FCDPA082-04	*Sclerocrangon boreas*	Crangonidae	Canada	Specimen	FolA–FolB	564
DQ882148	FCDPA086-04	*Sclerocrangon boreas*	Crangonidae	Canada	Specimen	FolA–FolB	658
DQ882147	FCDPA088-04	*Sclerocrangon boreas*	Crangonidae	Canada	Specimen	FolA–FolB	659
DQ882150	FCDPA142-04	*Sergestes similis*	Sergestidae	Canada	Specimen	FolA–FolB	646
DQ882152	FCDPA143-04	*Sergestes similis*	Sergestidae	Canada	Specimen	FolA–FolB	648
DQ882151	FCDPA144-04	*Sergestes similis*	Sergestidae	Canada	Specimen	FolA–FolB	647
DQ882149	FCDPA133-04	*Sergestes similis*	Sergestidae	Canada	Specimen	FolA–FolB	658
DQ882154	FCDPA028-04	*Spirontocaris holmesi*	Hippolytidae	Canada	Specimen	CrustF1–FolB	657

Table A3 (*concluded*).

GenBank accession No.	Process ID	Species	Family	Country	Archive type	Primer pair[a]	Length
DQ882153	FCDPA119-04	*Spirontocaris holmesi*	Hippolytidae	Canada	Specimen	FolA–FolB	658
DQ882156	FCDPA029-04	*Spirontocaris lamellicornis*	Hippolytidae	Canada	Specimen	CrustF1–FolB	626
DQ882155	FCDPA030-04	*Spirontocaris lamellicornis*	Hippolytidae	Canada	Specimen	CrustF1–FolB	621
DQ882157	FCDPA031-04	*Spirontocaris lamellicornis*	Hippolytidae	Canada	Specimen	CrustF1–FolB	658
DQ882158	FCDPA077-04	*Spirontocaris phippsi*	Hippolytidae	Canada	Specimen	FolA–FolB	589
DQ882159	FCDPA084-04	*Spirontocaris phippsi*	Hippolytidae	Canada	Specimen	FolA–FolB	659
DQ882162	FCDPA120-04	*Spirontocaris sica*	Hippolytidae	Canada	Specimen	FolA–FolB	657
DQ882161	FCDPA121-04	*Spirontocaris sica*	Hippolytidae	Canada	Specimen	FolA–FolB	658
DQ882160	FCDPA122-04	*Spirontocaris sica*	Hippolytidae	Canada	Specimen	FolA–FolB	658
DQ882163	FCDPA085-04	*Spirontocaris spinus*	Hippolytidae	Canada	Specimen	FolA–FolB	659
DQ882164	FCDPA099-04	*Synalpheus* sp.	Alpheidae	Australia	Specimen	FolA–FolB	653
DQ882166	FCDPA100-04	*Synalpheus* sp.	Alpheidae	Australia	Specimen	FolA–FolB	658
DQ882165	FCDPA101-04	*Synalpheus* sp.	Alpheidae	Australia	Specimen	FolA–FolB	659
DQ882167	FCDPA103-04	*Synalpheus* sp.	Alpheidae	Australia	Specimen	FolA–FolB	658
DQ882168	FCDPA049-04	*Systellaspis braueri*	Oplophoridae	Canada	Tissue	FolA–FolB	654
DQ882171	FCDPA091-04	*Uca* sp.	Ocypodidae	Australia	Specimen	FolA–FolB	659
DQ882169	FCDPA104-04	*Uca* sp.	Ocypodidae	Australia	Specimen	FolA–FolB	658
DQ882170	FCDPA152-04	*Uca* sp.	Ocypodidae	Australia	Specimen	CrustF1–FolB	632

[a]Primer FolA corresponds to LCO1490; FolB corresponds to HCO2198.

Table A4. List of decapod specimens for which COI barcodes were acquired from GenBank, together with their family and sequence length.

GenBank accession No.	Species	Family	Length
AF283888	*Agononida incerta*	Galatheidae	657
AF283889	*Agononida incerta*	Galatheidae	657
AB128829	*Alvinocaris* sp.	Bresiliidae	657
AY465911	*Callinectes arcuatus*	Portunidae	657
AY465913	*Callinectes arcuatus*	Portunidae	657
AY465907	*Callinectes bellicosus*	Portunidae	657
AY465909	*Callinectes bellicosus*	Portunidae	657
AY363392	*Callinectes sapidus*	Portunidae	658
AY465915	*Callinectes sapidus*	Portunidae	657
AY682072	*Callinectes sapidus*	Portunidae	658
AY682073	*Callinectes sapidus*	Portunidae	658
AY682074	*Callinectes sapidus*	Portunidae	658
AY682075	*Callinectes sapidus*	Portunidae	658
AY682076	*Callinectes sapidus*	Portunidae	658
AY682077	*Callinectes sapidus*	Portunidae	658
AY682078	*Callinectes sapidus*	Portunidae	658
AY682079	*Callinectes sapidus*	Portunidae	658
AY616445	*Carcinus aestuarii*	Portunidae	498
AY616437	*Carcinus maenas*	Portunidae	498
AY616438	*Carcinus maenas*	Portunidae	498
AY616439	*Carcinus maenas*	Portunidae	498
AY616440	*Carcinus maenas*	Portunidae	498
AY616441	*Carcinus maenas*	Portunidae	498
AY616442	*Carcinus maenas*	Portunidae	498
AY616443	*Carcinus maenas*	Portunidae	498
AY616444	*Carcinus maenas*	Portunidae	498
AF493625	*Cherax crassimanus*	Parastacidae	600
NC_011243	*Cherax destructor*	Parastacidae	658
AF493622	*Cherax preissii*	Parastacidae	600
AF493623	*Cherax preissii*	Parastacidae	600
AF493618	*Cherax quinquecarinatus*	Parastacidae	600
AF493619	*Cherax quinquecarinatus*	Parastacidae	600
AF493620	*Cherax quinquecarinatus*	Parastacidae	600
AF493621	*Cherax quinquecarinatus*	Parastacidae	600
AF493626	*Cherax tenuimanus*	Parastacidae	600
AF493627	*Cherax tenuimanus*	Parastacidae	600
AF493628	*Cherax tenuimanus*	Parastacidae	600
AF493629	*Cherax tenuimanus*	Parastacidae	600
AF493630	*Cherax tenuimanus*	Parastacidae	600
AF493631	*Cherax tenuimanus*	Parastacidae	600
AF493633	*Engaeus strictifrons*	Parastacidae	600
AF105249	*Eriocheir formosa*	Varunidae	658
AF105250	*Eriocheir formosa*	Varunidae	658
AF516698	*Eriocheir formosa*	Varunidae	558
AF317326	*Eriocheir formosa*	Varunidae	576
AF516699	*Eriocheir hepuensis*	Varunidae	558
AF317327	*Eriocheir hepuensis*	Varunidae	576
AF317328	*Eriocheir hepuensis*	Varunidae	576
AF105245	*Eriocheir japonica*	Varunidae	658
AF105246	*Eriocheir japonica*	Varunidae	658
AF516700	*Eriocheir japonica*	Varunidae	558
AF317330	*Eriocheir japonica*	Varunidae	576
AF317331	*Eriocheir japonica*	Varunidae	576
AF516701	*Eriocheir leptognathus*	Varunidae	558
AF316537	*Eriocheir leptognathus*	Varunidae	576
AF317332	*Eriocheir rectus*	Varunidae	576
AF105247	*Eriocheir sinensis*	Varunidae	658

Table A4 (*continued*).

GenBank accession No.	Species	Family	Length
AF105248	*Eriocheir sinensis*	Varunidae	658
AF435113	*Eriocheir sinensis*	Varunidae	588
AF435114	*Eriocheir sinensis*	Varunidae	588
AF435115	*Eriocheir sinensis*	Varunidae	588
AF435116	*Eriocheir sinensis*	Varunidae	588
AF435117	*Eriocheir sinensis*	Varunidae	588
AF435118	*Eriocheir sinensis*	Varunidae	588
AF435119	*Eriocheir sinensis*	Varunidae	588
AF516702	*Eriocheir sinensis*	Varunidae	558
AF317333	*Eriocheir sinensis*	Varunidae	576
AF317335	*Eriocheir sinensis*	Varunidae	576
AF317336	*Eriocheir sinensis*	Varunidae	576
AF493634	*Euastacus bispinosus*	Parastacidae	600
AF516703	*Gaetice depressus*	Varunidae	555
AF317339	*Gaetice depressus*	Varunidae	573
AF493632	*Geocharax falcata*	Parastacidae	600
AF317340	*Hemigrapsus sanguineus*	Varunidae	573
AF192875	*Jasus edwardsii*	Palinuridae	513
AF192877	*Jasus edwardsii*	Palinuridae	507
AF339473	*Jasus edwardsii*	Palinuridae	642
AF192882	*Jasus lalandii*	Palinuridae	513
AF192883	*Jasus verreauxi*	Palinuridae	518
AF399976	*Liberonautes latidactylus*	Potamonautidae	540
AF510695	*Macrobrachium australiense*	Palaemonidae	608
AF510696	*Macrobrachium australiense*	Palaemonidae	609
AF510697	*Macrobrachium australiense*	Palaemonidae	609
AF510699	*Macrobrachium australiense*	Palaemonidae	609
AF510702	*Macrobrachium australiense*	Palaemonidae	609
AF510704	*Macrobrachium australiense*	Palaemonidae	606
AF510705	*Macrobrachium australiense*	Palaemonidae	609
AF510706	*Macrobrachium australiense*	Palaemonidae	608
AF510708	*Macrobrachium australiense*	Palaemonidae	608
AF510709	*Macrobrachium australiense*	Palaemonidae	609
AF283885	*Munida rhodonia*	Galatheidae	657
AF283886	*Munida rhodonia*	Galatheidae	639
AF283887	*Munida rubrodigitalis*	Galatheidae	657
AF483156	*Pagurus acadianus*	Paguridae	525
AF483159	*Pagurus armatus*	Paguridae	521
AF483157	*Pagurus bernhardus*	Paguridae	525
AF483118	*Pagurus longicarpus*	Paguridae	642
AF483122	*Pagurus longicarpus*	Paguridae	642
AF483124	*Pagurus longicarpus*	Paguridae	642
AF483127	*Pagurus longicarpus*	Paguridae	642
AF483140	*Pagurus longicarpus*	Paguridae	642
AF483142	*Pagurus longicarpus*	Paguridae	642
AF483143	*Pagurus longicarpus*	Paguridae	642
AF483144	*Pagurus longicarpus*	Paguridae	642
AF483151	*Pagurus longicarpus*	Paguridae	642
AF483153	*Pagurus longicarpus*	Paguridae	642
AF483158	*Pagurus ochotensis*	Paguridae	525
AF483160	*Pagurus pollicaris*	Paguridae	525
AF483161	*Pagurus pollicaris*	Paguridae	525
AF483163	*Pagurus pollicaris*	Paguridae	525
AF483164	*Pagurus pollicaris*	Paguridae	525
AF483165	*Pagurus pollicaris*	Paguridae	525
AF483166	*Pagurus pollicaris*	Paguridae	525
AF483167	*Pagurus pollicaris*	Paguridae	525
AF483169	*Pagurus pollicaris*	Paguridae	525

Table A4 (*concluded*).

GenBank accession No.	Species	Family	Length
AF483170	*Pagurus pollicaris*	Paguridae	525
AF483171	*Pagurus pollicaris*	Paguridae	525
AJ640116	*Palaemon longirostris*	Palaemonidae	658
AF339452	*Panulirus argus*	Palinuridae	642
AF339453	*Panulirus cygnus*	Palinuridae	642
AF339454	*Panulirus echinatus*	Palinuridae	642
AF339456	*Panulirus guttatus*	Palinuridae	642
AF339457	*Panulirus homarus homarus*	Palinuridae	642
AF339458	*Panulirus homarus megasculpta*	Palinuridae	642
AF339459	*Panulirus inflatus*	Palinuridae	642
AF339461	*Panulirus japonicus*	Palinuridae	641
AB071201	*Panulirus japonicus*	Palinuridae	658
AF339462	*Panulirus laevicauda*	Palinuridae	642
AF339463	*Panulirus longipes femoristriga*	Palinuridae	642
AF339464	*Panulirus longipes longipes*	Palinuridae	642
AF339465	*Panulirus marginatus*	Palinuridae	641
AF339467	*Panulirus ornatus*	Palinuridae	636
AF339466	*Panulirus pascuensis*	Palinuridae	642
AF339468	*Panulirus penicillatus*	Palinuridae	642
AF339469	*Panulirus polyphagus*	Palinuridae	642
AF339470	*Panulirus regius*	Palinuridae	642
AF339471	*Panulirus stimpsoni*	Palinuridae	642
AF339472	*Panulirus versicolor*	Palinuridae	642
AF534894	*Paratya australiensis*	Atyidae	633
AF534898	*Paratya australiensis*	Atyidae	633
AF534901	*Paratya australiensis*	Atyidae	633
AF534902	*Paratya australiensis*	Atyidae	633
AY308079	*Paratya australiensis*	Atyidae	561
AY308080	*Paratya australiensis*	Atyidae	561
AY308085	*Paratya australiensis*	Atyidae	561
AY308086	*Paratya australiensis*	Atyidae	561
AY308101	*Paratya australiensis*	Atyidae	561
AY308104	*Paratya australiensis*	Atyidae	561
NC_002184	*Penaeus monodon*	Penaeidae	658
NC_005037	*Portunus trituberculatus*	Portunidae	658
AF399979	*Potamonautes lividus*	Potamonautidae	538
AF474366	*Procambarus acutus*	Cambaridae	658
AF346400	*Ranina ranina*	Raninidae	642
AF283869	*Raymunida cagnetei*	Galatheidae	657
AF283870	*Raymunida cagnetei*	Galatheidae	657
AF283871	*Raymunida cagnetei*	Galatheidae	657
AF283872	*Raymunida confundens*	Galatheidae	657
AF283873	*Raymunida confundens*	Galatheidae	657
AF283874	*Raymunida dextralis*	Galatheidae	657
AF283875	*Raymunida elegantissima*	Galatheidae	657
AF283876	*Raymunida elegantissima*	Galatheidae	657
AF283877	*Raymunida elegantissima*	Galatheidae	657
AF283878	*Raymunida elegantissima*	Galatheidae	657
AF283879	*Raymunida elegantissima*	Galatheidae	657
AF283880	*Raymunida elegantissima*	Galatheidae	657
AF283881	*Raymunida elegantissima*	Galatheidae	656
AF283882	*Raymunida erythrina*	Galatheidae	657
AF283883	*Raymunida erythrina*	Galatheidae	657
AY288292	*Raymunida formosanus*	Galatheidae	654
AF283884	*Raymunida insulata*	Galatheidae	657
AF399974	*Sudanonautes africanus*	Potamonautidae	528
AF516704	*Varuna litterata*	Varunidae	558
AF317343	*Varuna litterata*	Varunidae	573

Table A5. List of specimens of the genus *Gammarus* (Amphipoda) used for calculation of within- and among-species distances displayed in Table 1.

Process ID	Species	Country
FCGA011-04	*Gammarus aequicauda*	Italy
FCGA012-04	*Gammarus aequicauda*	Italy
FCGA041-04	*Gammarus chevreuxi*	Portugal
FCGA042-04	*Gammarus chevreuxi*	Portugal
FCGA043-04	*Gammarus chevreuxi*	Portugal
FCGA044-04	*Gammarus chevreuxi*	Portugal
FCGA027-04	*Gammarus crinicornis*	Belgium
FCGA029-04	*Gammarus crinicornis*	Belgium
FCGA045-04	*Gammarus crinicornis*	Portugal
FCGA054-04	*Gammarus duebeni*	Iceland
FCGA059-04	*Gammarus duebeni*	Iceland
FCGA072-05	*Gammarus duebeni*	Iceland
FCGA073-05	*Gammarus duebeni*	Iceland
FCGA074-05	*Gammarus duebeni*	Iceland
FCGA034-04	*Gammarus insensibilis*	Portugal
FCGA035-04	*Gammarus insensibilis*	Portugal
FCGA036-04	*Gammarus insensibilis*	Portugal
FCGA033-04	*Gammarus insensibilis*	Portugal
FCGA028-04	*Gammarus locusta*	Belgium
FCGA030-04	*Gammarus locusta*	Belgium
FCGA031-04	*Gammarus locusta*	Belgium
FCGA032-04	*Gammarus locusta*	Belgium
FCGA001-04	*Gammarus locusta*	Portugal
FCGA037-04	*Gammarus locusta*	Portugal
FCGA038-04	*Gammarus locusta*	Portugal
FCGA039-04	*Gammarus locusta*	Portugal
FCGA040-04	*Gammarus locusta*	Portugal
FCGA048-04	*Gammarus oceanicus*	Iceland
FCGA049-04	*Gammarus oceanicus*	Iceland
FCGA050-04	*Gammarus oceanicus*	Iceland
FCGA051-04	*Gammarus oceanicus*	Iceland
FCGA067-05	*Gammarus oceanicus*	Iceland
FCGA068-05	*Gammarus oceanicus*	Iceland
FCGA070-05	*Gammarus oceanicus*	Iceland
FCGA071-05	*Gammarus oceanicus*	Iceland
FCGA016-04	*Gammarus oceanicus*	Poland
FCGA017-04	*Gammarus oceanicus*	Poland
FCGA018-04	*Gammarus oceanicus*	Poland
FCGA019-04	*Gammarus oceanicus*	Poland
FCGA056-04	*Gammarus oceanicus*	Canada, Churchill
FCGA047-04	*Gammarus oceanicus*	Canada, Churchill
FCGA062-05	*Gammarus oceanicus*	Canada, Churchill
FCGA063-05	*Gammarus oceanicus*	Canada, Churchill
FCGA023-04	*Gammarus oceanicus*	Canada, Rimouski
FCGA025-04	*Gammarus oceanicus*	Canada, Rimouski
FCGA026-04	*Gammarus oceanicus*	Canada, Rimouski
FCGA002-04	*Gammarus* PS 1	Bulgaria
FCGA003-04	*Gammarus* PS 1	Bulgaria
FCGA004-04	*Gammarus* PS 1	Bulgaria
FCGA005-04	*Gammarus* PS 1	Bulgaria
FCGA006-04	*Gammarus* PS 1	Bulgaria
FCGA007-04	*Gammarus* PS 2	Bulgaria
FCGA008-04	*Gammarus* PS 2	Bulgaria
FCGA009-04	*Gammarus* PS 2	Bulgaria
FCGA010-04	*Gammarus* PS 2	Bulgaria

Table A5 (*concluded*).

Process ID	Species	Country
FCGA013-04	*Gammarus salinus*	Poland
FCGA014-04	*Gammarus salinus*	Poland
FCGA015-04	*Gammarus salinus*	Poland
FCGA076-05	*Gammarus salinus*	Poland
FCGA077-05	*Gammarus salinus*	Poland
FCGA078-05	*Gammarus salinus*	Poland
FCGA079-05	*Gammarus salinus*	Poland
FCGA080-05	*Gammarus salinus*	Poland
FCGA081-05	*Gammarus tigrinus*	Poland
FCGA082-05	*Gammarus tigrinus*	Poland
FCGA083-05	*Gammarus tigrinus*	Poland
FCGA084-05	*Gammarus tigrinus*	Poland
FCGA020-04	*Gammarus zaddachi*	Poland
FCGA021-04	*Gammarus zaddachi*	Poland

Note: Data from F.O. Costa and P.D.N. Hebert, unpublished data. PS, provisional species.

Table A6. List of specimens of the genus *Daphnia* (Cladocera) used for calculation of within- and among-species distances displayed in Table 1.

GenBank accession No.	Species	Length
AY323083	*Daphnia dadayana*	630
AY323084	*Daphnia dadayana*	630
AY323085	*Daphnia dadayana*	630
AY323086	*Daphnia dadayana*	630
AY323087	*Daphnia dadayana*	630
AY323088	*Daphnia dadayana*	630
AY323089	*Daphnia dadayana*	630
AY323090	*Daphnia dadayana*	630
AY323091	*Daphnia dadayana*	630
AY323092	*Daphnia dadayana*	630
AY323093	*Daphnia dadayana*	630
AY323094	*Daphnia dadayana*	629
AY323095	*Daphnia dadayana*	619
AY323096	*Daphnia dadayana*	630
AY323097	*Daphnia dadayana*	630
AY323098	*Daphnia dadayana*	630
AY323071	*Daphnia gessneri*	630
AY323072	*Daphnia laevis*	630
AY323073	*Daphnia laevis*	630
AY323074	*Daphnia menucoensis*	630
AY323075	*Daphnia menucoensis*	630
AY323076	*Daphnia menucoensis*	630
AY323077	*Daphnia menucoensis*	629
AY323078	*Daphnia menucoensis*	630
AY323079	*Daphnia menucoensis*	630
AY323080	*Daphnia menucoensis*	630
AY323081	*Daphnia menucoensis*	630
AY323082	*Daphnia menucoensis*	630
AY323123	*Daphnia ornithocephala*	629
AY323124	*Daphnia ornithocephala*	629
AY323125	*Daphnia ornithocephala*	629
AY323126	*Daphnia parvula*	630
AY323070	*Daphnia peruviana*	630
AF308971	*Daphnia similis*	645
AY323121	*Daphnia similis*	630
AY323048	*Daphnia* sp. 1 SA-2003	630
AY323049	*Daphnia* sp. 1 SA-2003	630
AY323050	*Daphnia* sp. 1 SA-2003	630
AY323051	*Daphnia* sp. 1 SA-2003	628
AY323052	*Daphnia* sp. 1 SA-2003	630
AY323053	*Daphnia* sp. 1 SA-2003	630
AY323054	*Daphnia* sp. 1 SA-2003	630
AY323055	*Daphnia* sp. 1 SA-2003	630
AY323056	*Daphnia* sp. 1 SA-2003	630
AY323057	*Daphnia* sp. 1 SA-2003	630
AY323058	*Daphnia* sp. 1 SA-2003	630
AY323059	*Daphnia* sp. 2 SA-2003	630
AY323060	*Daphnia* sp. 2 SA-2003	630
AY323061	*Daphnia* sp. 2 SA-2003	630
AY323062	*Daphnia* sp. 2 SA-2003	630
AY323063	*Daphnia* sp. 2 SA-2003	630
AY323064	*Daphnia* sp. 2 SA-2003	630
AY323065	*Daphnia* sp. 3 SA-2003	630
AY323066	*Daphnia* sp. 3 SA-2003	629
AY323067	*Daphnia* sp. 3 SA-2003	630

Table A6 (*concluded*).

GenBank accession No.	Species	Length
AY323068	*Daphnia* sp. 3 SA-2003	630
AY323069	*Daphnia* sp. 3 SA-2003	630
AY323122	*Daphnia* sp. 4 SA-2003	628
AY323099	*Daphnia spinulata*	630
AY323100	*Daphnia spinulata*	630
AY323101	*Daphnia spinulata*	630
AY323102	*Daphnia spinulata*	630
AY323103	*Daphnia spinulata*	630
AY323104	*Daphnia spinulata*	630
AY323105	*Daphnia spinulata*	630
AY323106	*Daphnia spinulata*	630
AY323107	*Daphnia spinulata*	630
AY323108	*Daphnia spinulata*	630
AY323109	*Daphnia spinulata*	630
AY323110	*Daphnia spinulata*	630
AY323111	*Daphnia spinulata*	630
AY323112	*Daphnia spinulata*	630
AY323113	*Daphnia spinulata*	630
AY323114	*Daphnia spinulata*	628
AY323115	*Daphnia spinulata*	630
AY323116	*Daphnia spinulata*	630
AY323117	*Daphnia spinulata*	630
AY323118	*Daphnia spinulata*	630
AY323119	*Daphnia spinulata*	630
AY323120	*Daphnia spinulata*	630

Note: Data adapted from Adamowicz et al. (2004).