A molecular phylogeny of marine amphipods in the herbivorous family Ampithoidae

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Ampithoid amphipods dominate invertebrate assemblages associated with shallow-water macroalgae and seagrasses worldwide and represent the most species-rich family of herbivorous amphipod known. To generate the first molecular phylogeny of this family, we sequenced 35 species from 10 genera at two mitochondrial genes [the cytochrome c oxidase subunit I (COI) and the large subunit of 16 s (LSU)] and two nuclear loci [sodium–potassium ATPase (NAK) and elongation factor 1-alpha (EF1)], for a total of 1453 base pairs. All 10 genera are embedded within an apparently monophyletic Ampithoidae (Amphitholida, Ampithoe, Biancolina, Cymadusa, Exampithoe, Paragrubia, Peramphithoe, Pleonexes, Plumithoe, Pseudoamphithoides and Sunamphitoe). Biancolina was previously placed within its own superfamily in another suborder. Within the family, single-locus trees were generally poor at resolving relationships among genera. Combined-locus trees were better at resolving deeper nodes, but complete resolution will require greater taxon sampling of ampithoids and closely related outgroup species, and more molecular characters. Despite these difficulties, our data generally support the monophyly of Ampithoidae, novel evolutionary relationships among genera, several currently accepted genera that will require revisions via alpha taxonomy and the presence of cryptic species.

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Introduction

Amphipod crustaceans in the family Ampithoidae Boeck 1871 dominate invertebrate assemblages associated with shallow-water macroalgae and seagrasses worldwide (Conlan 1982; Poore et al. 2008) and represent the most species-rich family of herbivorous amphipod known (209 species described in 15 genera, Horton & De Broyer 2014). These amphipods are ‘insect-like’ herbivores (Hay et al. 1987) in that they are locally abundant, small relative to the hosts that they use for both habitat and food, and can sometimes have strongly negative impacts on macrophyte dynamics in the field (Chess 1993; Graham 2002; Mukai & Iijima 1995; Reynolds et al. 2012, but see Carpenter 1986; Poore et al. 2009). Despite featuring in hundreds of studies on the ecology of nearshore habitats, and proposed hypotheses about the influence of phylogeny on their feeding and behaviour (Poore et al. 2008), there are no molecular-based phylogenies that compare relationships within and among the genera of Ampithoidae. Molecular studies have been used to describe relationships among lineages for a small subset of species within ampithoid genera (McCarty & Sotka 2013; Sotka et al. 2003; Pilgrim & Darling 2010; Kim et al. 2012). Based on morphological traits, the family Ampithoidae is a robust monophyletic group.
defined by the outer ramus of uropod 3 having 1–2 recurved, robust apical setae (Barnard & Karaman 1991; Myers & Lowry 2003).

Within the family, only one major evolutionary hypothesis has been offered, with Conlan (1982) generating a phylogeny for 12 genera based on 27 morphological characters (Fig. 1). In that work, *Amphitholina* Ruffo, 1953 was ‘strongly separated’ from the other genera within the Ampithoidae, supporting previous work by Barnard (1972) and Myers (1974) which recognized Amphitholinae as a distinct subfamily. The remaining ampithoid genera were grouped into three clades: the genus *Pleonexes*; genera which have their first gnathopod with oblique palms (e.g. *Ampithoe* Leach, 1814); and genera with transverse palms on gnathopod 1 (e.g. *Exampithoe* K.H. Barnard, 1926). The genus *Pseudoampithoides* Ortiz, 1976 was an exception, as it shares several characters with the transverse-palmed genera, but has oblique palms on gnathopod 1. The phylogeny proposed by Conlan (1982) was based on phenetic methods, and there have been no family-level phylogenies produced using either cladistic or molecular methodology. Furthermore, most of the genera with the Ampithoidae are currently characterized by few morphological characters, and in at least one case, by continuous traits that overlap between genera. For example, the presence of a mandibular palp separates the genera *Peramphithoe* Conlan and Bousfield, 1982 and *Sunampithoe* Bate, 1857, but is both present and absent within the genus *Exampithoe* (Myers & Lowry 2003). These uncertainties, and the likelihood that many morphological traits in the amphipods are homoplasious (Hurt et al. 2013; Lowry & Myers 2013), suggest that a phylogeny based on molecular data could lead to new insights into evolution in this group.

In this study, we used sequences from two mitochondrial genes [the cytochrome c oxidase subunit I (COI) and the large subunit of 16 s (LSU)] and two nuclear loci [sodium–potassium ATPase (NAK) and elongation factor 1-alpha (EF1)] to construct the first molecular phylogeny of 10 ampithoid genera, including nine of the 12 genera from Conlan (1982).

**Methods**

**Taxon sampling**

Amphipods from the family Ampithoidae were collected by hand from shallow-water habitats worldwide (Table S1) and placed into 95% denatured ethanol. Our collections included 35 species from 10 genera from Europe, North America, South America, Asia and Australasia. For two

![Fig. 1](image-url) The phylogeny of Ampithoidae proposed by Conlan (1982) based on phenetic analysis of 27 morphological characters. Genera with a black dot were sequenced in this study.
species with wide geographic distributions, we separately analysed samples from more than one region (Ampithoe ramosi from Brazil and Hawaii, A. valida from east coast USA, west coast USA and Japan). For A. tarasovi, we separately analysed two colour morphs recognized by researchers in Japan (Hiroyuki Ariyama, personal communication). We also collected Biancolina japonica from the family Biancolinidae J.L Barnard, 1972 as this genus has been closely associated with the Ampithoidea in past taxonomic treatments (Stebbing 1906; Ruffo 1953).

Amphipods were identified to genus and species before extraction of DNA. To be confident of species identifications, male specimens were typically used as the characters involved in species-level identification of amphipods are frequently those associated with secondary sexual characters, in particular the morphology of the enlarged second gnathopod. All samples were identifiable to described taxa except for three novel species; one from Sunampitoe and two from Exampitoe. In most cases, we had to destructively sample entire amphipods during extraction. Other individuals from the same samples were held as voucher specimens and have been deposited in the Australian Museum. Table S1 includes GenBank accession numbers and voucher identification for all species sampled. Three other species from the suborder Senticaudata were used as outgroups: Gammarus mucronatus (infraorder Gammarida; family Gammaridae), Parbyale burwaiensis (infraorder Talitrida; family Hyalidae) and Melita plumulosa (infraorder Hadziida; family Melitidae).

Generating DNA sequences
Amphipod DNA was extracted using Qiagen (Limburg, the Netherlands) DNeasy Blood and Tissue kit. We amplified loci using the primers listed in Table S2. Gene regions were amplified using the following 20 μL recipe: 4 μL Promega GoTaq 5X Buffer (Promega, Madison, WI, USA), 0.75 mM total dNTP, 3.75 mM MgCl2, 0.25 μM (5 pmol) of forward and reverse primers, 1 μg/μL Bovine Serum Albumin (NEB, Ipswich, MA, USA), 1 unit of GoTaq polymerase and approximately 10 to 50 ng of DNA. Standard PCR conditions were used for 16 s and COI with the following cycles and temperatures: 95 °C for 60 s, 90 °C for 30 s, 50 °C for 1 min, 72 °C for 1 min, for 30–35 cycles. For EF1, the above PCR conditions were used except for 52 °C annealing temperature instead of 45 °C. For NAK, the above conditions were used or a touchdown PCR protocol to increase the success of PCR amplification.

Unincorporated primers and dNTPs were removed using Antarctic phosphatase and exonuclease I (NEB) using the following protocol: in a 12–17 μL reaction, 10–15 μL of PCR product was combined with 1 μL of exonuclease I (20 units/μL), 1 μL of Antarctic phosphatase (5 units/μL). This reaction mixture was then incubated at 37 °C for 15 min and 80 °C for 15 min. Each of these ‘clean’ reactions was then quantified using a NanoDrop spectrophotometer (Thermo-Scientific, Waltham, MA) and diluted to 40 ng/μL. These reactions were then sent to Macrogen USA (Rockville, MD, USA) for sequencing. Forward and reverse sequences were assembled and edited using Geneious 4.8 (http://www.geneious.com, Kearse et al. 2012). Any sequences with lower than 80% quality scores within Geneious were discarded and resequenced. Heterozygotes were scored as ambiguous with codes of R or Y depending on purine or pyrimidine changes.

Analyses
Alignment. For COI, EF1 and NAK, all sequences were aligned using CLUSTAL-X (Larkin et al. 2007) and visually inspected. In cases in which more than one individual of a terminal node was sequenced, we generated a majority consensus sequence using CONSENSUS in the package SEQIN (v. 3.0-7c) (Charif & Lobry 2007). For LSU (or the mitochondrial 16 s) sequences, we made the consensus sequence first and then generated alignments with multiple gap and extension penalties (10/5, 10/1, 5/5, 5/1, 2/2, 2/1) following MacDonald et al. (2005). A visual inspection of the resulting trees indicated that the topology was broadly similar, and we pursued analysis using the 10/5 alignment because of its consistency with other loci across most nodes. We inferred phylogenies using three or four loci for each taxa. Sequences were concatenated using APE (Paradis et al. 2004) and manipulated using SEAVIEW (Gouy et al. 2010) and MESQUITE (Maddison & Maddison 2015).

Substitution saturation. We assessed substitution saturation of codon positions in our protein-coding loci (COI, EF1 and NAK) as implemented in DAMBE (Xia 2013). At codon positions 1 and 2 of COI, I)* (a measure of substitution saturation) was significantly lower than I)* (a critical value determined from computational simulation) while I)* and I)* were indistinguishable at codon position 3. This pattern indicates substantial saturation at codon position 3, and we removed this locus for subsequent analyses. By these same criteria, all codon positions at EF1 and NAK were not saturated.

Phylogenetic analysis – single-locus. We randomly subsetted 10 taxa and used jModelTest2 (Darriba et al. 2012) to evaluate the best model of evolution (as determined by AIC criteria) for each locus (Table S3). We analysed all individuals for which we had data at a given locus, and thus, the number of individuals differed across loci. Single-locus data sets were analysed with a Bayesian search in
Bayesian searches were implemented in BEAST (Rambaut et al. 2010) using 50 million generations, sampling every 10 000 generations. After removal of 10 000 of 50 000 replicates. Support values were summarized using SPLITSTREE (Drummond et al. 2012) on the CIPRES server (Miller et al. 2016 Royal Swedish Academy of Sciences) and visualized using FIGTREE (Rambaut 2014) and APE. See Figs S1–S4 for individual trees.

Phylogenetic analysis – multilocus. Multilocus analyses included all species (n = 40 amphipod + 3 outgroup taxa) for which at least three or more loci were sequenced (1453 bp total). Because incorporating a partitioning strategy may fundamentally alter phylogenetic inference, we inferred phylogenies using no partitions (P1), a partition by genome (P2A), by locus type (P2B) and by locus (P4; Table S3). Bayesian searches were implemented in BEAST (Drummond et al. 2012) on the CIPRES server (Miller et al. 2010) using 50 million generations, sampling every 10 000 generations. After removal of 10 000 of 50 000 trees, log likelihood (LnL) was consistent across two independent runs at each locus and ESS values exceeded 1000. A Bayesian-factor analysis (or an absolute difference in the harmonic means of lnL; (Kass & Raftery 1995) indicated that P2A was the most appropriate model (Table S3).

We also pursued bootstrap ML analyses with RAxML (Stamatakis 2014) using RAxML (Silvestro & Michalak 2011) on all (un)partitioned data sets using GTR+GAMMA for each partition, two independent runs and 10 000 bootstrap replicates. Support values were summarized using SPLITSTREE (Sukumaran & Holder 2010), and trees were visualized using PHYLOCLOSE (Heibl 2013) and APE.

Results

Phylogeny of the Ampithoidae

Single-locus phylogenies (Figs S1–S4) showed strong support for the monophyly of the Ampithoidae at three of four loci. The exception was at LSU where a polytomy included all amphipod species plus an outgroup of Gammarus and Parhyale species. When all data were combined (Fig. 2; Fig. S5), there was high posterior probability (100%) and ML-bootstrap support (100%) of a monophyletic Ampithoidae.

Within the Ampithoidae, the monophyly of Cymadusa Savigny 1816 and Exampithoe was well supported in both multilocus (Fig. 2) and some single-locus phylogenies. In contrast, we found no statistical support for a distinction between Perampithoe and Sunampithoe. The genus Bianolina Della-Valle, 1893, most recently placed in the monotypic family Bianolinidae, is clearly nested within the Ampithoidae.

We detected two major clades within the most speciose genus in the family, the currently-accepted genus Ampithoe. One clade, termed here the Ampithoe ‘south’ clade, represents the most ancestral lineage of Ampithoidae. An analysis of occurrence data from the Global Biodiversity Information Facility indicates that the ‘south’ clade dominates temperate to tropical Pacific Oceans, as well as the Indian, Mediterranean and Atlantic Oceans. The Ampithoe ‘north Pacific’ clade is a more derived clade whose species occur within the northern, cold-temperate Pacific Ocean (Table 1; Fig. 3). The exception to this pattern is Ampithoe valida which occurs in both the north Pacific and Atlantic (see Discussion).

We also found several broadly dispersed species that represent a complex of multiple cryptic species or subspecies. Specimens identified as Ampithoe ramondi collected from Hawaii and Spain are likely different species, as the former clusters with A. kava (from Australia) and the latter with A. plumulosa (from Ecuador) and A. longimanus (from the US east coast). Japanese specimens identified as A. tasavski ‘light’, A. tasavski ‘dark’ and A. lacertosus formed a single monophyletic clade.

Systematics

Order Ampipoda Latreille, 1816

Suborder Corophiidea Leach, 1814

Infraorder Corophiida Leach, 1814

Superfamily Corophioidea Leach, 1814

Family Ampithoidae Boeck, 1871

Diagnosis (unaltered from Myers & Lowry, 2003). Labium outer plate with or without distal notch or excavation. Uropod three outer ramus with two recurved robust setae, or with one small, straight or weakly curved robust seta. Telson cusps present or absent.

Subfamily Ampithoinae Boeck, 1871

Diagnosis (unaltered from Myers & Lowry, 2003). Mandible palp 3-articulate or absent. Labium outer plate with distal notch or excavation. Uropod three outer ramus with two recurved robust setae or with one small, straight or weakly curved robust seta.

Generic composition. Ampithoe Leach, 1814; Ampithoides Kossmann, 1880; Ampitholinia Ruffo, 1953; Austrathoe Peart, 2014; Bianolina Della-Valle, 1893; Cymadusa Savigny, 1816; Macropisthopus K. H. Barnard, 1916; Paradusa Ruffo, 1969; Paragrapsia Chevreux, 1901; Paranexes Peart, 2014; Perampithoe Conlan and Boufield, 1982; Plumiphtoe Barnard and Karaman, 1991; Pseudampithoides Ortiz, 1976;
Pseudopleonexes Conlan and Bousfield, 1982; Sunamphitoe Bate, 1857.

Remarks. Transferring Biancolina back to Ampithoidae subsumes its previous status as a monotypic superfamily. This is a substantial shift in taxonomic status and taxon relationships, transferring between suborders. Such extreme transitions can be expected for highly derived monotypic groups. Convergent morphology is evident in several genera of amphipods that are known to burrow inside algal tissues (Mejaes et al. 2015). Note that five genera were not sampled as part of the molecular analyses in this study (Ampitholina, Ampithoe, Cymadusa, Exampithoe, Paragrubia, Peramphithoe, Plumithoe, Pseudoamphithoides and Sunamphitoe).

Discussion
Ampithoid amphipods are numerically dominant members of shallow marine ecosystems worldwide and can have important ecological roles as herbivores. Here, we generated the first molecular treatment of phylogenetic relationships within the Ampithoidae using 1453 base pairs across two nuclear and two mitochondrial loci for 35 amphithoid species across 10 genera. Overall, single-locus trees were generally poor at resolving relationships among genera. Combined-locus trees were better at resolving deeper nodes, but complete resolution will require greater taxon sampling of both amphithoids and closely related outgroup species and more molecular characters. Despite these issues, the existing data set suggests the monophyly of Ampithoidae, novel evolutionary relationships among genera, several currently accepted genera that will require revisions via alpha taxonomy and the presence of cryptic species.

Ampithoid genera
We sequenced eight of the currently accepted genera within Ampithoidae (Ampitholinia, Ampitoxe, Cymadusa, Exampithoe, Paragrubia, Peramphithoe, Plumithoe, Pseudoamphiboides and Sunamphitoe), and all are embedded within a monophyletic Ampithoidae (Fig. 4). We note, however, that our
phylogeny was rooted with outgroup species that are not Corophiida, the infraorder to which Ampithoidae belongs. We suggest that future work should attempt to confirm monophyly using outgroup species that are more closely related to Ampithoidae.

Our data also indicate that the genus *Biancolina* should be considered within the family Ampithoidae. Based on morphological characters only, *Biancolina* was positioned within the talitrids most recently by Lowry & Myers (2013). This taxonomic placement follows a highly mobile

Table 1 *Ampithoe* clade distributions. *Ampithoe* species were delineated by molecular phylogeny into north Pacific or south clades. Hypothesized clades for other *Ampithoe* species are based on reported geographic distributions. Citation: GBIF.org (4th June 2015) GBIF Occurrence Download http://doi.org/10.15468/dl8i5go

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<td>dalli</td>
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<td>Japan</td>
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Hypothesized North Pacific clade species: kussakini, lindbergi, rubricatoides, sectimana, simulans.

Hypothesized Basal clade species (Atlantic, Mediterranean, Indian or South Pacific distributions): boiana, brevipes, cinera, cookana, ferox, gammaroides, guaspare, helleri, hiana, hinatore, hirsutimanus, hyalos, kaneohi, katee, kergueleni, kuala, kulafi, marcuzzii, meganae, ningaloo, parakava, platycera, pollux, pseudongana, rachanoi, rodel, roly, rozema, rubricata, tea, ulladulla, virescens, Waialua.

Ambiguous distribution (Southern California; Baja): humeralis, plumulosa.

Fig. 3 The distribution of *Ampithoe*-‘north Pacific’ clade (red circles) and *Ampithoe*-‘south’ clade (blue dots). The cosmopolitan species *Ampithoe valida* is not included. See Table 1 for data sources.
history of the higher classification of this genus, with previous workers each acknowledging the distinctness of the group. 

Biancolina was originally placed with the Dexamiminidae (Della-Valle 1893). However, Stebbing (1906) synonymized the species with Amphibolina cuniculus, which first placed Biancolina as a junior synonym within the family Amphitoidae. This placement of Biancolina within Amphibolina was subsequently rejected by all further workers.

With the description of a second species, Biancolina was included in the then-newly-established Prophiliantidae Nicholls, 1939. The genus was again placed in association with the Amphitoidae by Ruffo (1953) and further supported by Myers (1974), however, between this time, Barnard (1969) had proposed it as part of the Eophiliantidae Sheard, 1936. Biancolina was then established within the monotypic family Biancoliidae by Barnard (1972), with remarks on its close relationships to Amphibolina (Amphitoidae) and Wandelia Chevreux 1906 (Eophiliantidae), later expanded upon in Barnard & Karaman (1991). A phylogeny of the Talitroida by Serejo (2004) included Biancolina-Paragrubia, the sister taxa Pseudoamphithoides-Infracardiidae (Paragrubia edgari); Just 1977 (Pseudoamphibolidae incurvaria); Lowry 1974 (Biancolina brassicaephala); Myers 1974 (Amphibolina cuniculus); Poore and Lowry 1997 (Amphiboloe caddi, Exampiboloe kutti, Perampiboloe parmerong, Plunithoequadrimana).

The name ‘Plunithoe’ was proposed by Bate (1856), was considered a nomen nudem until 1857 when sufficient description of the group was provided. Conlan’s (1982) cluster analysis (Fig. 2) recognized the group at the generic level. Barnard & Karaman (1991) considered Plunithoe as a subgenus within Amphiboloe for species with preevalve pereopods and the presence of large recurved hooks on the telson. They included the species A. poipu; A. aptos; and A. belleri in this subgenus, as well as A. auriculata, where the telsonic hooks are considered intermediately developed. This study was able to include sequences from one of these taxa (A. poipu) and find it within the Amphiboloe-‘south’ clade, which itself appears to be basal to Amphitoidae. This suggests that the Amphiboloe poipu may represent a species from a group with telsonic hooks that is basal to the entire family. Testing this hypothesis will require more thorough sampling of the species in this group.

Relationships among genera

Our current understanding of generic relationships within the Amphitoidae is shown in Fig. 4 (nodes with >0.95 posterior probability). There is a large sister clade that includes Cymadusa, the sister taxa Pseudoamphibolidae-Amphibolina and Sunampiboloe/Perampiboloe. There is strong support for a Plunithoe-Exampiboloe sister clade. The phylogenetic relationships between these two clades, Biancolina, Paragrubia, and Amphiboloe-‘north Pacific’ remains uncertain. The basal status of Amphiboloe-‘south’ has strong Bayesian support, and its separation from the remainder of the amphitoid taxa had 78% support from ML bootstrap (Fig. 2; Fig. S5).

Our molecular phylogeny contrasts in several ways with the morphological-based phylogeny of Conlan (1982) (Fig. 1). Conlan (1982) suggested Cymadusa and Amphiboloe are sister taxa, but our molecular phylogeny clusters Cymadusa with Amphibolina, Perampiboloe, Sunampiboloe and Pseudoamphithoides. Exampiboloe clusters with these last three taxa by Conlan (1982), but our phylogeny suggests that Exampiboloe and Plunithoe are sister taxa. Finally, Conlan (1982) and Myers & Lowry (2003) suggest that Amphibolina is a basal taxa, while our phylogeny suggests it is derived.

Among our most surprising findings, the cosmopolitan and most speciose genus, Amphiboloe, is composed of two significantly supported clades that are split geographically between the north Pacific and other areas. We predict that
species we did not sample but with a distribution localized to the north Pacific will likely also fall into the Ampithoe-
north Pacific’ clade (Table 1). This includes A. kussakini,
A. lindbergi, A. rubricatoides and A. simulans. We also pre-
dict that any other species with Atlantic, Mediterranean,
Indian or south Pacific distributions will be part of the
Ampithoe-’south’ clade (see Table 1 for species listing).

While it is possible that these clades reflect two different
genera, this remains a preliminary hypothesis that will
require greater resolution of the deeper nodes that deline-
cate clades. Moreover, our preliminary investigation of
existing morphological traits in the DELTA database
(Hughes et al. 2008) to world amphipod genera and species –
including mouthpart and external morphological charac-
ters – identified only a single synapomorphic character that
divides these two groups. The mandibular palp article 3 is
clavate (medially or distally broad, proximally more narrow)
in the Ampithoe-’north Pacific’ while Ampithoe-’south’
species have a palp that is slender (of uniform breadth).
Identification of only a single morphological character is
surprising given the significant genetic support for splitting
these clades. We note that these two groups are not
consistent with the split of Ampithoe species by Conlan &
Bousfield (1982) into a cluster of three ‘subgroups’ based
on their morphological survey of north-eastern Pacific spe-
cies (1-A. lacertos, A. valida, A. plumulosa; 2-A. kussakini,
A. volki, A. sectimana; 3-A. rubricatoides, A. dalli, A. simu-
lans). Instead, we find that A. plumulosa is embedded within
the Ampithoe-’south’ clade and A. valida, A. sectimana and
A. dalli are Ampithoe-’north Pacific’.

Molecular data also force a reevaluation of morphologi-
cal traits that were used to define other genera. For exam-
ple, Peramphithoe and Sunamphitoe were historically split by
the presence and absence of a mandibular palp, respec-
tively. Our molecular analyses indicate that this single trait
is not a good generic character and that these genera
should be synonymized. These genera remain united by
the outer ramus of uropod one and the broad lower lip of
inner lobes (as in Conlan 1982). The sister-taxa relation-
ship of Plumithoe and Exampithoe is united by a feeble to
poorly developed mandibular palp. These morphological
traits are, however, tentative, and a more thorough sam-
ping of species within and across these particular genera
and examination of their morphological and molecular
traits will be required to resolve these issues.

Cryptic species
Within species, there is clear evidence that cryptic, ancient
lineages are present among some amphipods. As an example,
previous studies suggest that Ampithoe valida is composed of at
least three deep genetic mitochondrial lineages (Pilgrim &
Darling 2010). Consistent with their data, we find an Atlantic
specimen (AmpVaN) diverged from Pacific (AmpVaG and
AmpVaB) using all molecular data (Fig. 2, Fig. S5) and with
significant support at one of four single loci (COI; Fig. S2).

Given that Ampithoe valida is embedded within the Ampithoe-
north Pacific’ clade, it is likely that the A. valida complex
originated with other Ampithoe-’north Pacific’ species and
then dispersed (via natural means) into the Atlantic where it
then diverged at COI (Pilgrim & Darling 2010). There is also
evidence that some Atlantic lineages have reinvaded the Paci-
fic coastline (Pilgrim & Darling 2010), but this hypothesis will
require future sampling. As with other amphipod taxa with
cosmopolitan distributions, molecular data can be effective in
identifying cryptic speciation (e.g. Pilar Cabezas et al. 2013).

Deep divergence among lineages at the population, sub-
species or species level also occurs within Ampithoe longi-
mana (McCarty & Sotka, 2013; Sotka et al. 2003),
Peramphithoe femorata (E. Sotka and M. Thiel, unpub-
lished), Peramphithoe tea (E. Sotka and J. Long, unpub-
lished), Ampithoe ramondii and Ampithoe tarasovi (this study).

Detailed taxonomic research on the morphology of species
in this family has also demonstrated that species thought to
be cosmopolitan are comprised of species complexes (e.g.
Cymadusa filosa, Peart 2004). Their brooding lifestyle and
close association with benthic macrophytes would suggest
that localized distributions are likely for species in this fam-
ily; however, several species are known to occur on drifting
macroalgae (e.g. Sunamphitoe pelagica, Stoner & Greening
1984; Peramphithoe femorata Rothausker et al. 2011) and fur-
ther work is needed to understand likely patterns of dispers-
al in this group.

Conclusions
The molecular phylogeny of 10 genera of herbivorous
 amphipods in the family Amphipodidae revealed the inclu-
sion of Biancolina as a monotypic genus, representing a sig-
nificant transition from previous works which assess the
taxa as a superfamily within a different suborder. Surpris-
ingly, this study also revealed cryptic genus-level lineages
in the currently accepted Ampithoe that will require more
species sampling and morphological and molecular data.

The phylogeny represents a profoundly incongruent
conclusion with previous morphological generic concepts
within the Amphipodidae. Among the amphipods, this is not
unique to Amphipodidae. Morphological and molecular traits
are commonly incongruent, for example in the Gammarus
species complex (Hou et al. 2007; Weiss et al. 2014), the
Lake Baikal amphipod radiation (MacDonald et al. 2005),
and among subterranean freshwater taxa (Finston et al.
2007). This is not surprising, as there are relatively few
molecular studies of the higher levels of classification of the
Amphipoda (Lowry & Myers 2013; Myers & Lowry
2003; Barnard & Karaman 1991) and amphipod
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References

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

*Fig. S1–S5*. Phylogenies using individual locus data sets (S1–S4) and a comparison of ML and Bayesian tree in Figure S5. Numbers indicate posterior support from MrBayes. Species abbreviations are noted in Table S1.

**Table S1.** List of species, collection locations, seaweed hosts, abbreviation and locus sequenced with GenBank Accession numbers.

**Table S2.** Primers for polymerase chain reactions. F1/R1 amplified NAK for all species except for *Peramphithoe* (F2/R2) and *Cymadusa* (F3/R3).

**Table S3.** Partitioning strategy during phylogenetic analyses.