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AN EVALUATION OF CRYPTIC LINEAGES OF *IDOTEA BALTHICA* (ISOPODA: IDOTEIDAE): MORPHOLOGY AND MICROSATELLITES

John P. Wares, Sarah Daley, Regina Wetzer, and Robert J. Toonen

(JP) Department of Genetics, University of Georgia, Athens, Georgia 30602, U.S.A.
(to whom correspondence should be addressed: jpwares@uga.edu);
(SD, RJT) Hawai'i Institute of Marine Biology, University of Hawai'i at Manoa, Hawai'i, U.S.A.
(RW) Natural History Museum of Los Angeles County, Los Angeles, California, U.S.A.

ABSTRACT

Idotea balthica has long been studied for intraspecific variation at the phenotypic and ecological level. Recent work found deep genetic divergences among several regional populations of this species in the North Atlantic. Here we report development of microsatellite loci for *Idotea* and use these newly developed markers to evaluate population structure among the mitochondrial clades. We also provide a detailed morphological evaluation, including SEM description of relevant traits in one of the more divergent groups. Phenotypically, the examined clades do not differ significantly, though individuals from populations in Virginia (U.S.A.) have more setose pereiopods than European and other museum specimens. The preliminary microsatellite data presented here suggest that these markers should be of utility for a variety of small-scale ecological and behavioral analysis in some related *Idotea* species. Although microsatellites are inappropriate for species designations, there is significant differentiation among mitochondrial lineages indicating that nuclear loci may show similar differentiation as mtDNA among regions. Together the subtle morphological variation and microsatellite data suggest that additional nuclear gene sequencing and reproductive studies are warranted to determine the taxonomic status of these regional forms.

INTRODUCTION

Studies of marine biodiversity are becoming complicated by the realization that there are far more evolutionarily distinct lineages of species than can be recognized using phenotypic measures (Knowlton, 2000; Palumbi, 1994; Wares and Castañeda, 2005). This diversity is often called 'cryptic' but it can now be taken for granted that many coastal species are far more fragmented than previously described (Wares, 2001). Much of this description of genealogical diversity has so far involved inference from mtDNA sequence data, which due to properties of effective population size and rapid substitution rate (Avise et al., 1987) often exhibits patterns of differentiation among geographic regions faster than comparable nuclear markers.

Identifying new species using molecular data as a primary inference is a novel approach to taxonomy, and has led to increased debate in recent years associated with the 'DNA barcoding' approach (Hebert et al., 2003; Moritz and Cicero, 2004; Rubinoff and Holland, 2005). Often molecular data will guide taxonomists in more intense morphological scrutiny of populations (Wares, 2001; McFadden et al., 2006; Pitombo and Burton, in press), but in some cases the only characters available to distinguish 'species' may be these genetic characters. In general, the controversy over the utility of these data isn't whether such characters are valid, but that such strong inference is attempted based on data from a single, small portion of a much larger genome.

So in attempts to use molecular data to delineate species, we should be more quantitative in our description of what constitutes a species. Hudson and Coyne (2002) outlined approaches to quantify the time of divergence among populations based on the frequency of reciprocally monophyletic marker sequences, and noted attempts to quantify reproductive isolation (the biological species concept) based on this frequency. Moritz et al. (1996) and others have suggested, at least for the purposes of biodiversity and conservation studies, that concordance between mitochondrial and nuclear genes is necessary to delineate species or "evolutionarily significant units" (ESUs). To the extent that we can combine these approaches and utilize multilocus data to understand whether or not multiple species—potentially with different ecologies or other relevant life history traits (Bowen, 1999; Pringle et al., 2005)—exist may be very useful for quantifying levels and patterns of biodiversity in natural communities.

Wares (2001) considered patterns of ecological and genealogical variation in the amphi-Atlantic isopod *Idotea balthica* (Pallas, 1772). While this species has long been studied for patterns of color polymorphism and genetic variation in the form of isozyme data (Bulnheim and Fava, 1982; Guarino et al., 1993; Jormalainen et al., 1995), Wares (2001) showed that at least 4 divergent monophyletic lineages of *I. balthica* can be resolved using mitochondrial sequence data. These distinct clades (with strong bootstrap support) were found in Virginia (U.S.A.), Nova Scotia (Canada), Iceland, and an amphi-Atlantic lineage that may have expanded to North America following Pleistocene glaciation (Wares and Cunningham, 2001). The strong divergence among some of these clades coincides with novel lineages found in other species, including a large number of divergent genetic patterns among species of the marine community around Long Island Sound (Wares, 2002).

Here we revisit the problem of taxonomic and genealogical diversity in north Atlantic *I. balthica* by evaluating morphological variation in specimens of these distinct clades as well as providing additional nDNA information. We present newly developed microsatellite loci that amplify

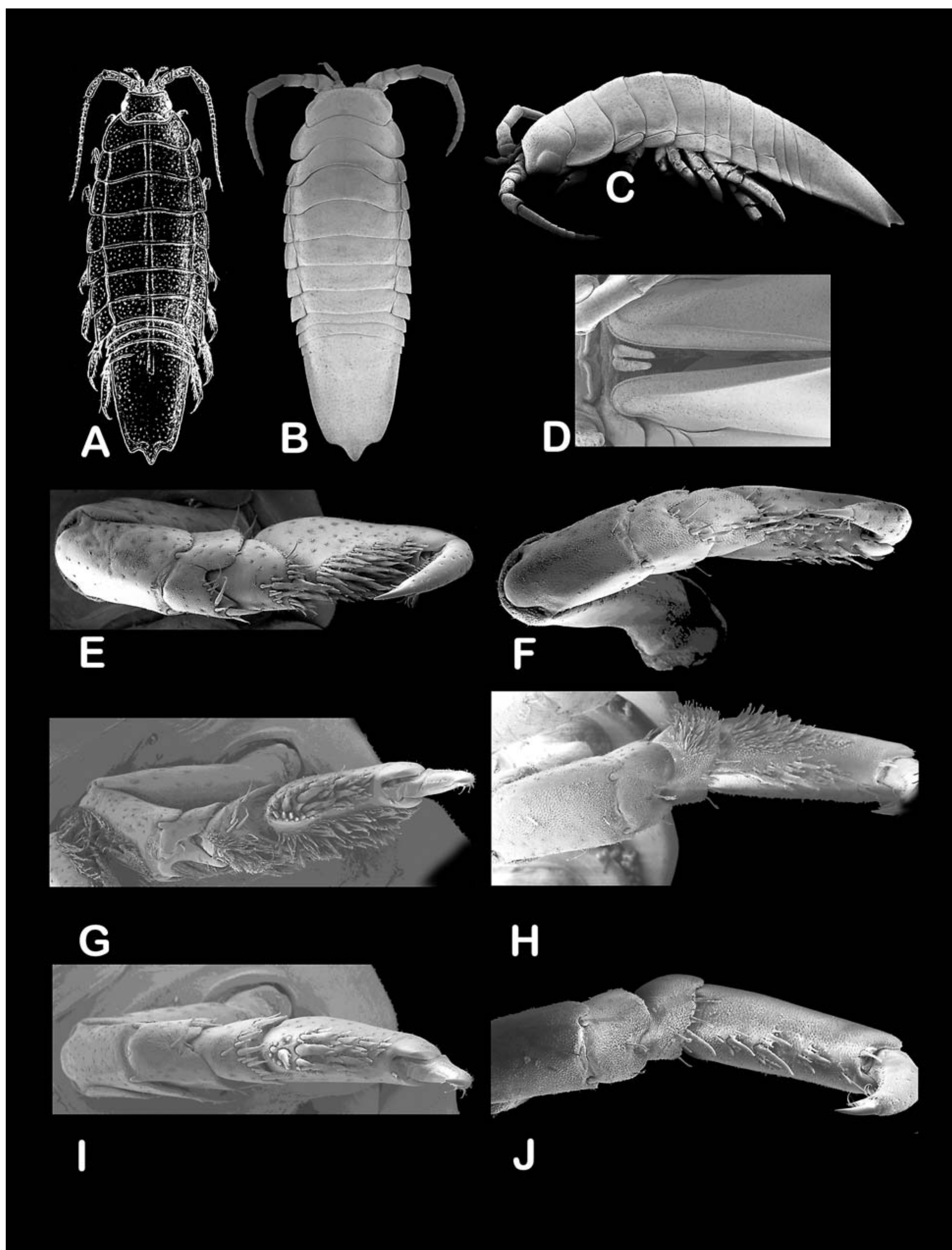

Fig. 1. *Idotea balthica* (Pallas, 1772). A, from Sars, 1899 pl. 32; B, male dorsal view, Iceland; C, male lateral view, Iceland; D, male penes, Virginia; E, left pereiopod 1, Virginia; F, right pereiopod 1, Iceland; G, right pereiopod 2, Virginia; H, left pereiopod 2, Iceland; I, right pereiopod 3, Virginia; J, left pereiopod, 3 Iceland. Iceland and Virginia specimens are from lots RW02.063, RW02.072, respectively. Locality data in Appendix A.

Table 1. Primers for microsatellite amplification in *Idotea balthica*. Successful PCR reactions required 4.6 µl H_2O, 1 µl Invitrogen 10× buffer, 0.8 µl dNTPs, 0.4 µl $MgCl_2$, 1 U *Taq*, 1 µl of each primer (10µM concentration) and 1µl of DNA template (5-10ng/µl).

Primer	Sequence	[Primer], µM	Annealing temp.	Repeat motif
*Iba*2F	TATGCGTAGAAGCGGCTCTG	1.25	56	$(AC)_{18}$ $(AT)_{31}$
*Iba*2R	ACCATTCAGGCACTAGGCAATA			
*Iba*4F	AAGCTGCTTTTGCCTTTTTG	5	58	$(AT)_{38}$
*Iba*4R	ACTTCCCAATCCAAGCTCAG			
*Iba*6F	AGTGGGTTCTTTGCCTCTTG	5	50	$(TC)_{23}$
*Iba*6R	TTCGATAAAGAAACGTCAAAAGC			
*Iba*15F	GGTGTAAGGAGGCATGGAGA	2.5	55	$(AG)_{20}$
*Iba*15R	TGGTGCACGTTCTGTAATCC			

in these four lineages of *I. balthica* and should be applicable to a number of population and migration questions in this species. These data show distinctions among the four lineages, but sample sizes and statistical analysis of these data will have to be increased to fully understand patterns of evolution among these lineages and their implications for the historical and adaptive causes of isolation. We also present SEM photos of relevant traits, which indicate that some populations may vary phenotypically, but in subtle ways.

METHODS

The genus *Idotea* Fabricius, 1798 presently contains 27 species worldwide and is known mostly from cold temperate waters. Some species are poorly described and may be junior synonyms of valid taxa, and still others await reassignment to other genera (G. Poore, personal communication). In the North Atlantic, Baltic, and Mediterranean Seas ten species are recognized: *Idotea emarginata* (Fabricius, 1793), the type species (originally designated as *Cymothoa*) occurs in the northeast Atlantic, intertidal to 20 m; *I. granulosa* Rathke, 1843, Norway, intertidal; *I. neglecta* G. O. Sars, 1897, Norway, intertidal to 40 m; *I. linearis* (Linnaeus, 1766), northeast Atlantic, Mediterranean, intertidal to shallow water; *I. phosphorea* Harger, 1873, northwest Atlantic, intertidal; *I. whymperi* Miers, 1881, north Atlantic; *I. metallica* Bosc, 1802, cosmopolitan, pelagic on floats, buoys, rafting vegetation; *I. balthica* (Pallas, 1772), northeast Atlantic, 20-34 m; *I. chelipes* (Pallas, 1766), northeast Atlantic, Mediterranean, intertidal to 6 m; and *I. pelagica* Leach, 1815, northeast Atlantic, intertidal. Only *I. balthica*, *I. chelipes*, and *I. pelagica* conform to the generic redescription of the type species *I. emarginata* (Poore and Lew Ton, 1993), are considered closely related, and are further discussed below.

Idotea balthica is about 3 times as long as broad with a smooth dorsal surface. The pleotelson is distinctly straight-sided with a tridentate distal apex, the middle tooth is conically produced, lateral teeth are much shorter than middle tooth. *I. pelagica* is distinguished from *I. balthica* by its comparatively short and stout body, scarcely 3 times as long as broad. *I. emarginata* is oblong to oval, scarcely 3 times as long as broad. The pleotelson posterior margin is abruptly truncated, slightly emarginated with lateral corners projecting distinctly beyond posterior margin. *Idotea chelipes* is about 5 times as long as broad with coxal plates deeply indented, pleotelson vaulted, and apex tapering to a blunt point.

The widely distributed *I. balthica* had been previously recognized with subspecies which are now considered synonyms of this species (G. Poore, personal communication). Differences in morphological characters, growth allometries, rates of sexual development, and geographic distribution (Tinturier-Hamelin, 1963) had been used to distinguish: *Idotea balthica balthica* Dall (Baltic Sea), brackish; *I. balthica basteri* Audouin, 1826 (Mediterranean), marine; *I. balthica tricuspidata* Desmarest, 1825 (Atlantic), marine; *I. balthica stagnea* (Tinturier-Hamelin, 1960) (French Mediterranean), brackish. Our morphological investigation examined specimens from the North Atlantic (Iceland and Denmark), U.S.A. (Massachusetts and Virginia), several specimens from the Black Sea (Romania), and a single specimen from Argentina.

Table 2. Information on each marker by regional sample of *I. balthica* defined by mtDNA clades. For each marker sample size (N), number of alleles (Na), observed heterozygosity (Ho), expected heterozygosity (He), inbreeding coefficient (F), and *p* value for deviation from HWE are indicated.

Pop	*Iba*2	*Iba*4	*Iba*6	*Iba*15
Iceland				
N	8	5	8	9
Na	13	6	14	4
Ho	1.000	0.200	0.750	0.444
He	0.906	0.820	0.922	0.574
F	−0.103	0.756	0.186	0.226
p	n.s.	< 0.01	< 0.01	n.s.
Atlantic				
N	18	10	17	18
Na	21	20	24	4
Ho	0.778	1.000	0.824	0.333
He	0.931	0.950	0.948	0.557
F	0.164	−0.053	0.131	0.402
p	n.s.	n.s.	n.s.	n.s.
Virginia				
N	6	5	6	
Na	9	7	10	2
Ho	0.833	0.400	0.833	0.600
He	0.861	0.840	0.889	0.420
F	0.032	0.524	0.062	−0.429
p	n.s.	< 0.01	n.s.	n.s.
NS				
N	2	2	2	2
Na	4	4	4	1
Ho	1.000	1.000	1.000	0.000
He	0.750	0.750	0.750	0.000
F	−0.333	−0.333	−0.333	—
p	n/a	n/a	n/a	n/a

Specimens of *I. balthica* for genetic and morphological analysis were obtained from sites representing the four lineages from Wares (2001), including the Virginia Clade (Gloucester Point, Virginia; n = 6), Iceland clade (Hvassahraun, Iceland; n = 8), Nova Scotian clade (Antigonish, Nova Scotia, Canada; n = 2) and the "Atlantic" lineage (Damariscotta, Maine, U.S.A.; n = 7; Antigonish, Nova Scotia, Canada; n = 5; Galway, Ireland; n = 3; and Roscoff, France; n = 3). These were immediately preserved in 90% ethanol upon collection and were compared for morphological traits with representative specimens from museum collections (Appendix A) using both light microscopy and SEM.

Genetic Marker Development

A microsatellite library was developed from genomic DNA isolates using the protocol of Toonen (1997). Genomic DNA was fragmented with restriction enzymes (*Sau*3A*I* and *MboI*) and size-selected via gel purification to obtain fragments approximately 400-750 bp in size. DNA fragments were ligated into BlueScript KS+ plasmid vector and transformation was via electroporation. Positive-insert colonies were screened via chemiluminescent probing with appropriate di-, tri- and tetranucleotide oligonucleotides (Toonen, 1997). Approximately 40 positive colonies were sequenced and screened for simple sequence repeat motifs visually using CHROMAS 2.24 (Technelysium Pty, Ltd). Primers for putative microsatellite regions were developed using PRIMER3 (Rozen and Skaletsky, 2000).

Primers were initially optimized for polymerase chain reaction conditions via screening on 3% agarose gels, followed by direct optimization of fluorescently-labeled primers on an ABI 3100XL genotyping system. Markers that did not amplify consistently given 3 touchdown PCR protocols (dropping annealing temperatures from: 68° to 60°, 62° to 55°, and 60° to 50°) were discarded, as were loci that exhibited no variation across the test populations collected for this study. Remaining markers were screened for reliability and amplification in a larger number of DNA isolates, including individuals of *I. metallica* Bosc, 1802, *Pentidotea*

resecata (Stimpson, 1857), and *P. wosnesenskii* (Brandt, 1851 and were scored using ABI GeneMapper, with fragment size calibrated with a ROX500 size standard. Marker data was analyzed using GenAlEx v.6 (Peakall and Smouse, 2006) to calculate allele frequencies within and between each mitochondrial lineage, along with relevant diversity statistics, heterozygosity, fit to Hardy-Weinberg expectations (HWE) and F-statistics. Loci were also examined with MicroChecker (van Oosterhout et al., 2004) for scoring difficulty such as band stutter or null alleles.

Results

Morphological analysis of contemporary populations in North America and Europe (see Appendix A) indicate phenotypic variation among mtDNA lineages from specimens of *I. balthica* representing the European and probable amphi-Atlantic lineage of this species. Figure 1 compares the Sars (1899) line drawing of *I. balthica* with a recently collected Iceland specimen. Sars illustrated North East Atlantic collections comparable to our Iceland collections. Regrettably Sars' specimen is unavailable for re-examination. Three distinct morphological differences between Sars' specimen and the more recently collected Iceland specimens are noteworthy: cephalon width, antenna flagellum length, and the length and width of the pleotelson. Morphologically more striking are the differences of Virginia (VIMS) and Iceland specimens in regard to pereiopods 1-3 with the VIMS specimens clearly more setose than specimens from others localities (Fig. 1). Penes characteristics are often taxonomically useful and figured here for a VIMS specimens (Fig. 1D). Penes have not been previously figured for *I. balthica*. The Iceland collection had no adult males. Large adult males collected at VIMS reach lengths of 17 mm. Antenna 1 extends to antenna 2-peduncle article 2 anterior margin. The Massachusetts specimens, which are large adult males up to 21 mm in length, have setose pereiopods 1 and 2, but are less setose overall compared to Virginia specimens. However, the Massachusetts specimens were collected in 1882, and the difference in setae may be an artifact of age and preservation.

Iceland and Denmark specimens are very similar; both have comparatively little body setation, and pereiopods are weakly setose. Antenna 1 is short, extending to the antenna 2 peduncle article 1 posterior margin. In both Icelandic and Danish samples, the pleotelson posterior margin is strongly tridentate with the middle tooth prominently acute. Large mature males (Danish specimens) have longer penes than males from other localities examined. No mature males were available from Iceland. Males reach lengths up to 19 mm, whereas gravid females reach 12 mm. Setation of Black Sea specimens is similar to Iceland/Denmark specimens. In all these specimens, the pleotelson posterior margin middle tooth is short and blunt. Males reach lengths of 19 mm, whereas gravid females reach 9 mm in length. A single adult male from Argentina (24 mm in length) has a similar setation pattern to Virginia specimens, with fairly long antenna 1, and somewhat shorter pleotelson posterior margin middle tooth compared to Iceland and Denmark specimens.

Morphologically cryptic lineages defined by mtDNA (Wares, 2001) also showed significant differentiation at the four nuclear microsatellite loci we developed (Table 1). These loci were developed specifically for *I. balthica* (source material from VIMS), and our preliminary study shows that they do not co-amplify in Pacific isopods (*Pentidotea resecata* and *P. wosnesenskii*), but two of the loci do amplify in Atlantic populations of the cosmopolitan species *I. metallica*. However, locus *Iba*6 appears monomorphic in the small sample ($n = 4$) of *I. metallica* we tested, and we found only two alleles using locus *Iba*15 in these samples. Despite the small sample sizes of our initial screening, all four microsatellite loci are polymorphic in each of the four mitochondrial lineages of *I. balthica*, except for the small sample ($n = 2$) from Nova Scotia which appears monomorphic for *Iba*15.

Although our sample sizes are too small to make conclusive statements, there are no shared alleles at three of the four microsatellite loci between the Virginia and Nova Scotia lineages (*Iba*2, *Iba*4, *Iba*6) and the Nova Scotia and Iceland lineages (*Iba*4, *Iba*6, *Iba*15). In general, there is considerable overlap of amplified fragment sizes among mitochondrial lineages at each locus, and no allele is present at frequency > 0.25. Thus, additional samples may well fill in the distribution and reveal any shared alleles among the lineages. Regardless, the current multilocus microsatellite data suggest at least modest differentiation among the mitochondrial lineages. Calculated F_{st} values are > 0.12 among all pairs of lineages except for the Iceland and amphi-Atlantic lineages (F_{st} 0.062). However, with such high heterozygosities (Table 2), these F_{st} values are close to the maximum that can be calculated with such loci (Hedrick, 2005). Recoding the data to estimate the standardized F_{st} (Meirmans, 2006), indicates that differentiation among mtDNA lineages is substantial, and many of these values are close to the maximum that can be calculated for these loci, i.e., approaching an F_{st} of 1.0.

Analysis of our preliminary data using MicroChecker suggested some deviation from HWE may be due to null alleles. While there was no evidence for scoring error, large allele dropout, or null alleles, i.e., homozygote excess, for most primer-population combinations, there was significant ($P < 0.05$) homozygote excess or other indication of null alleles for *Iba2* in the New England population; *Iba4* in the Iceland and Virginia populations; *Iba6* in the Iceland and New England populations; and *Iba15* in the New England population (with too few data to analyze the Nova Scotia samples). The sporadic nature of these results suggests that increased sampling could resolve many of these potential errors.

Discussion

The value of the codominant microsatellite markers developed here is great for this brooding species, as we now have better tools for studies of trait heritability, kin interactions, regional migration, and evolutionary effects of life history traits. Although our sample sizes here are quite small, making our inference only preliminary, it is clear that these loci harbor considerable variation.

The current data are inconclusive with regard to the presence of distinct species nested within the North Atlantic *I. balthica*. However, the monophyletic mtDNA lineages reported by Wares (2001), the subtle morphological variation seen among some pairs of clades, and the genetic differentiation among lineages at microsatellite loci appear

to merit further study to understand what maintains the geographic separation of such lineages. Theoretical approaches (Hudson and Coyne, 2002) can identify species' separation times based on the frequency of reciprocal monophyly among loci compared between lineages. Our current result suggests either a recent divergence, sufficient to generate a robust pattern in mtDNA but not in morphological characters, or the potential for local adaptation or other forces to affect only mitochondrial diversity (Rand, 2001; Schizas et al., 2001; Foltz, 2003). While intracellular infection by *Wolbachia* Hertig, 1936 (see Bouchon et al., 1998) may influence mitochondrial diversity patterns in some terrestrial isopods, there is no evidence for such infection in *Idotea* (Bouchon et al., 1998; Wares, unpublished data). Furthermore, the genetic differentiation among the lineages in this initial microsatellite data set is quite high, and suggests that sequencing is warranted to examine phylogenetic signal in nuclear genes for direct comparison to the mitochondrial data set.

Although the uncorrected estimates of F_{st} (> 0.12) are unremarkable, the loci developed for this study have very high heterozygosity (Table 2), and several studies have shown that the maximum level of F_{st} that can be calculated is less than the average within population homozygosity (Hedrick, 2005; Meirmans, 2006). This is in part because of increased homoplasy at microsatellite loci with increased divergence time, suggesting that microsatellites may be positively misleading in terms of divergence patterns if the allelic range is broad and there is sufficient overlap among evolutionary lineages in this range (O'Reilly et al., 2004). Because of these issues, microsatellite length polymorphism data are clearly a poor basis for the evaluation of species, but the high degree of population differentiation among the mtDNA lineages discounts the likelihood of mitochondrial-specific explanations of this diversity and argues that additional studies on these regional differences are warranted. However, not all patterns reported by Wares (2001) are corroborated by the microsatellite data presented here. For example, mtDNA haplotype diversity was significantly lower in North American populations (Wares, 2001), but there is no such difference between regional diversities for these four microsatellite loci; all were equally polymorphic in both European and American regions.

Morphological variation was only slight among mitochondrial lineages, with the Virginia population having more setose pereiopods than other populations of *I. balthica*, but otherwise little obvious differentiation was found. Figure 1 illustrates greater setation in specimens from Virginia than the specimens from the Icelandic clade. While much previous work has catalogued the color polyphenisms in *I. balthica* (Bulnheim and Fava, 1982; Guarino et al., 1993; Merilaita, 2001), we have only anecdotal evidence that the Virginia population is also distinct in this regard (T. Bell, personal communication). Preliminary evidence also suggests that the Virginia population responds differently in terms of growth and reproduction than European populations when raised in a common-garden environment (Gutow, Franke, and Wares, unpublished data). Considering all the evidence, it is premature to consider taxonomic revision for this species and its cryptic lineages, but the current study only adds to the mounting evidence that a detailed evaluation of the regional differences in *I. balthica* is warranted. Sequence data from nuclear loci will be the next logical approach to this question, but to fully evaluate the specific status of different clades of *I. balthica*, a combination of behavioral and phylogenetic assays will be necessary.

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References

Avise, J. C., J. Arnold, R. M. Ball, E. Bermingham, T. Lamb, J. E. Neigel, C. A. Reeb, and N. C. Saunders. 1987. Intraspecific phylogeography: the mitochondrial DNA bridge between population genetics and systematics. Annual Review of Ecology and Systematics 18: 489-522.

Bouchon, D., T. Rigaud, and P. Juchault. 1998. Evidence for widespread *Wolbachia* infection in isopod crustaceans: molecular identification and host feminization. Proceedings of the Royal Society of London B 265: 1081-1090.

Bosc, L. A. G. 1802. Histoire Naturelle des Crustaces. In, G. L. L. Buffon (ed.), Histoire Naturelle de Buffon, classee d'apres le system de Linne par R. R. Castel. Paris.

Brandt, F. 1851. Krebse, pp. 145-147. In, A. T. von Middendorf (ed.), Reise in den aussersten Norden und Osten Sibiriens während—1843 und 1844—auf Veranstaltung der Kaiserlichen Academie der Wissenschaften zu St. Petersburg. Vol. 2, Part 1. St. Petersburg.

Bowen, B. W. 1999. Preserving genes, species, or ecosystems? Healing the fractured foundations of conservation policy. Molecular Ecology 8: S5-S10.

Bulnheim, H. P., and G. Fava. 1982. Color polymorphism and genetic variation in Idotea baltica populations from the Adriatic Sea and Baltic Sea. Genetica 59: 177-190.

Fabricius, J. C. 1793. Entomologia systematica emendata et aucta ... adjectis synonymis, locis, observationibus, descriptionibus. 4 vols., 1792-1794. Copenhagen.

Foltz, D. W. 2003. Invertebrate species with nonpelagic larvae have elevated levels of nonsynonymous substitutions and reduced nucleotide diversities. Journal of Molecular Evolution 57: 607-612.

Guarino, S. M., C. Gambardella, M. Ianniruberto, and M. de Nicola. 1993. Colour polymorphism in *Idotea baltica* from the Bay of Naples and its ecological significance. Journal of the Marine Biological Association of the United Kingdom 64: 21-33.

Harger, O. 1873. In, A. E. Verrill, S. I. Smith, and O. Harger (eds.), Catalogue of the Marine Invertebrate animals of the southern coast of New England, and adjacent waters. In, A. E. Verrill and S. I. Smith, Report upon the invertebrate animals of Vineyard Sound and adjacent waters, with an account of the physical features of the region. In, S. F. Baird, Report of Professor S. F. Baird, Commissioner of Fish and Fisheries, on the condition of the sea-fisheries of the south coast of New England in 1871 and 1872. Washington, D.C.: Government Printing Office.

Hebert, P. D. N., S. Ratnasingham, and J. R. de Waard. 2003. Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species. Philosophical Transactions of the Royal Society of London B 270S: 1-4.

Hedrick, P. W. 2005. A standardized genetic differentiation measure. Evolution 59: 1633-1638.

Hertig, M. 1936. The Rickettsia, *Wolbachia pipientis* (gen. et sp. n.) and associated inclusions of the mosquito, *Culex pipens*. Parasitology 28: 453-486.

Hudson, R. R., and J. A. Coyne. 2002. Mathematical consequences of the genealogical species concept. Evolution 56: 1557-1565.

Jormalainen, V., S. Merilaita, and J. Tuomi. 1995. Differential predation on sexes affects colour polymorphism of the isopod *Idotea baltica* (Pallas). Biological Journal of the Linnean Society, London 55: 45-68.

Knowlton, N. 2000. Molecular genetic analyses of species boundaries in the sea. Hydrobiologia 420: 73-90.

Leach, W. E. 1815. A tabular view of the external characters of four classes of animals, which Linnée arranged under Insecta, with the distribution of the genera composing three of these classes into orders, and description of several new genera and species. Transactions of the Linnean Society of London 11: 306-400.

Linnaeus, C. 1766. Systema Naturae per Regna tria Naturae, secundum classes, ordines, genera, cum characteribus, differentiis, synonymis, locis. Twelfth edition. pp. 533-1227. Vienna.

McFadden, C. S., S. C. France, J. A. Sanchez, and P. Alderslade. 2006. A molecular phylogenetic analysis of the Octocorallia (Coelenterata: Anthozoa) based on mitochondrial protein-coding sequences (ND2, msh1). Molecular Phylogenetics and Evolution 41: 513-527.

Meirmans, P. G. 2006. Using the AMOVA framework to estimate a standardized genetic differentiation measure. Evolution 60: 2399-2402.

Merilaita, S. 2001. Habitat heterogeneity, predation and gene flow: Colour polymorphism in the isopod, *Idotea baltica*. Evolutionary Ecology 15: 103-116.

Miers, E. J. 1881. Revision of the Idoteidae, a family of sessile-eyed Crustacea. Journal of the Linnean Society of London 16: 1-88.

Moritz, C. 1996. Uses of molecular phylogenies for conservation, pp. 203-216. In, P. Harvey, A. J. Leigh Brown, and J. Maynard Smith (eds.), New Uses for New Phylogenies, Oxford University Press, New York.

———, and C. Cicero. 2004. DNA Barcoding: promise and pitfalls. PLoS Biology 2: e354.

O'Reilly, P. T., M. F. Canino, K. M. Bailey, and P. Bentzen. 2004. Inverse relationship between Fst and microsatellite polymorphism in the marine fish, walleye pollock (*Theragra chalcogramma*): implications for resolving weak population structure. Molecular Ecology 13: 1799-1814.

Pallas, P. S. 1766. Miscellanea Zoologica, quibus novae imprimis atque obscurae animalium species describuntur et observationibus iconibusque illustrantur. Petrum van Cleef, Hague.

———. 1772. Spicilegia Zoologica, quibus novae imprimus et obscurae animalium species iconibus, descriptionibus atque commentariis illustrantur. Berlin.

Palumbi, S. R. 1994. Genetic divergence, reproductive isolation, and marine speciation. Annual Review of Ecology and Systematics 25: 547-572.

Peakall, R., and P. E. Smouse. 2006. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. Molecular Ecology Notes 6: 288-295.

Pitombo, F. B., and R. S. Burton. (in press). Taxonomy and biogeography of the *Chthamalus fissus* species group on the western coast of the Americas. Zootaxa.

Poore, G. C. B., and H. M. Lew Ton. 1993. Idoteidae of Australia and New Zealand (Crustacea: Isopoda: Valvifera). Invertebrate Taxonomy 7: 197-278.

Pringle, A., D. M. Baker, J. L. Platt, J. P. Wares, J. P. Latge, and J. W. Taylor. 2005. Cryptic speciation in a global and apparently asexual fungus, the human pathogen *Aspergillus fumigatus*. Evolution 59: 1886-1899.

Rand, D. M. 2001. The units of selection on mitochondrial DNA. Annual Review of Ecology and Systematics 32: 415-448.

Rathke, H. 1843. Beiträge zur Fauna Norwegens. Nova Acta Academiae Caesareae Leopoldino Carolinae 20: 1-264c.

Rozen, S., and H. Skaletsky. 2000. Primer3 on the WWW for general users and for biologist programmers, pp. 365-386. In, S. Krawetz and S. Misener (eds.), Bioinformatics Methods and Protocols: Methods in Molecular Biology. Humana Press, Totowa, NJ.

Rubinoff, D., and B. S. Holland. 2005. Between two extremes: Mitochondrial DNA is neither the panacea nor the nemesis of phylogenetic and taxonomic inference. Systematic Biology 54: 952-961.

Sars, G. O. 1897. An account of the Crustacea of Norway with short descriptions and figures of all the species. Vol. 2. Isopoda part V, VI. Idotheidae, Arcturidae, Asellidae, Ianiridae, Munnidae. Bergen Museum, Bergen, Norway, pp 81-116.

Schizas, N. V., G. T. Chandler, B. C. Coull, S. L. Klosterhaus, and J. M. Quattro. 2001. Differential survival of three mitochondrial lineages in a marine copepod exposed to a mixture of pesticides. Environmental Science and Technology 35: 535-538.

Stimpson, W. 1857. The Crustacea and Echinodermata of the Pacific shores of North America. Boston Journal of Natural History 6: 1-92.

Tinturier-Hamelin, E. 1960. Nouvelles recherches sur le polytypisme d'*Idotea baltica* (Pallas) (Isopode Valvifere): definition d'*I. b. stagnea* nov. ssp. Comptes Rendus des Seances de l'Academie des Sciences 250: 2606-2608.

———. 1963. Polychromatisme et determination genetique du sexe chez l'espece poltypique *Idotea baltica* (Pallas) (Isopodes Valivere). Cahiers de Biologie Marine 4: 473-591.

Toonen, R. J. 1997. Microsatellites for ecologists: Non-radioactive isolation and amplification protocols for microsatellite markers. Online resource, http://www2.hawaii.edu/~toonen/Msats/.

van Oosterhout, C., W. F. Hutchinson, D. P. M. Wills, and P. Shipley. 2004. Micro-checker: software for identifying and correcting genotyping errors in microsatellite data. Molecular Ecology Notes 4: 535-538.

Wares, J. P. 2001. Intraspecific variation and geographic isolation in Idotea balthica (Isopoda: Valvifera). Journal of Crustacean Biology 21: 1007-1013.

———. 2002. Community genetics in the northwestern Atlantic intertidal. Molecular Ecology 11: 1131-1144.

———, and C. W. Cunningham. 2001. Phylogeography and Historical Ecology of the North Atlantic Intertidal. Evolution 55: 2455-2469.

———, and A. E. Castañeda. 2005. Geographic range in *Chthamalus* along the west coast of North America. Journal of the Marine Biological Association, United Kingdom 85: 327-331.

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APPENDIX A: MATERIAL EXAMINED

Abbreviations are as follows: AHF, Allan Hancock Foundation; LACM, Natural History Museum of Los Angeles County; RW, Regina Wetzer; USNM, United States National Museum.

Iceland, Seltjarnes, 64.167°N 22.017°W, 7 October 2002. Station 6. Coll. Agnar Ingólfsson. RW02.063, 8 specimens without penes, also without oosteigites-no gravid females; 6 specimens with penes—largest ones also have longest penes, body to 13 mm.

USA, Virginia, Virginia Institute of Marine Sciences (VIMS), ~37.259°N ~76.496°W, 7 October 2002. Coll. Emmet Duffy. RW02.072, 2 largest individuals are males; the 2 small individuals are females with brood pouches just beginning to develop, male to 17 mm.

USA, Massachusetts, Woods Hole, U.S. Fish Commission, ~41.526°N ~70.674°W, 27 January 1882. USNM 3862. Coll. V. N. Edwards. RW06.157, males to 21 mm, specimens quite soft, not well preserved.

Argentina, Chubut Province, Peninsula Valdés, ~42.2°S ~64°W, 1 February 1974. USNM Acc. No. 319597. Coll. Jane Frick. RW06.158, 1 large male, 24 mm with slightly blunter pleotelson apical point than European specimens, fairly long antenna 1, quite setose pereiopods, similar to VIMS specimens.

Denmark, Vordingborg, off Vestenback Wood (Vestenbak Skov), 55.01°N 11.55°E, dredge, 8 m. 3 July 1922. Sta. 2625, AHF Cat. No. 1103-01, LACM 22-1.1. Coll. "Thor". RW06.159, very acute pleotelson, similar to Iceland specimens largest males with very long penes, males to 19 mm, largest female to 12 mm (3 of which are gravid females).

Black Sea, Romania, Constanta, sand beach, under stones covered with algae, *Mytilus*, 44.12°N 28.4°E, 2 July 1979. Coll. Ileana Negoescu. RW06.160, 7 males; 3 females of which 2 are gravid, females much smaller than males, apex of pleotelson not as acute as Iceland specimens; pereiopod and body setation weak and much like Iceland specimens largest males to 19 mm, largest female to 9 mm.