

Diversity and dispersal history of the talitrids (Crustacea: Amphipoda: Talitridae) of Bermuda

D. J. Wildish^a, S. R. Smith^b, T. Loeza-Quintana^c, A. E. Radulovic^d and S. J. Adamowicz^c

^aFisheries & Oceans Canada, Biological Station, New Brunswick, Canada; ^bBermuda Natural History Museum, Aquarium and Zoo, Flatt's, Bermuda; ^cDepartment of Integrative Biology & Biodiversity Institute of Ontario, University of Guelph, Guelph, Canada; ^dBiodiversity Institute of Ontario, University of Guelph, Guelph, Canada

ABSTRACT

Five taxa of talitrid amphipods were found in the archipelago of Bermuda, of which three were recorded there for the first time. Four of these are supralittoral wrack generalists: *Platorchestia monodi* BOLD:AAB3402, (a unique Molecular Operational Taxonomic Unit according to the Barcode Index Number system), a related species recognized by molecular methods, *Platorchestia platensis* BOLD:AAA2949, *Mexorchestia carpeniteri carpeniteri* BOLD:AAC1491 and *Tethorchestia antillensis*; and one a terrestrial leaf-litter generalist: *Talitroides alluaudi*. A key is provided to discriminate between the formally described talitrids of Bermuda. Dispersal mechanisms from the American continent to Bermuda were considered for all taxa based on species distributions along the North American Atlantic coast and also investigated by molecular methods, using genetic population differentiation and haplotype network analysis based on the barcode region of cytochrome *c* oxidase subunit I gene. For *P. monodi* BOLD:AAB3402 the genetic results suggest that some dispersal events occurred before human colonization of Bermuda but are equivocal about the source population and therefore the direction of dispersal. Some very recent synanthropic dispersal is possible with this species. For the other two species studied genetically, *P. platensis* BOLD:AAA2949 and *M. c. carpeniteri* BOLD:AAC1491, the small population samples analysed support dispersal to Bermuda from the American mainland, before human occupation of Bermuda, although the available sample size was limited for these species. The available limited direct, non-genetic evidence supports synanthropic transport for *Talitroides alluaudi*. *Platorchestia monodi* BOLD:AAB3402 is found in the same wrack habitat as *P. platensis* BOLD:AAA2949 on Bermuda, apparently without interbreeding. No evidence was found that driftwood specialist talitrids had become established in Bermuda.

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Introduction

At the time of the origin of talitrids (Crustacea: Amphipoda: Talitridae), some 110 million years ago (MYA) according to Bousfield (1984), the volcanic eruptions that first formed

the islands of Bermuda were also occurring (Rowe 1998). Volcanic activity ceased some 30 MYA, and in modern times the igneous rock has been overlain by sedimentary sandy limestone so that the average thickness (below current sea level) of sedimentary rock is 75 m (Rowe 1998). The present-day sedimentary limestone overlying the igneous rock is of Pleistocene origin, with a maximum age of 2 MYA (Rowe 1998). Fluctuations in sea level throughout the Pleistocene produced extensive modifications of number and size of the Bermuda islands, which resulted in extinctions and radiations of the terrestrial fauna (Olson and Hearty 2003; Olson et al. 2006; Hearty and Olson 2010), including birds, reptiles and molluscs. Hence, in geological terms the terrestrial, and presumably also the supralittoral marine, fauna of Bermuda is of relatively recent origin (< 2 MYA), and Bermuda may be regarded as a 'Darwinian' island in the sense used by Gillespie and Roderick (2002). That is to say, the terrestrial and littoral fauna colonized and radiated on the island *de novo* < 2 MYA. Events during the Pleistocene that could have impacted talitrids and caused extinctions were increases of sea level, notably during an interglacial ~ 0.4 MYA, during which much of present day Bermuda was below sea level (Olson et al. 2006).

The present-day Bermudian archipelago has a land surface of only 53 km², and the nearest continental land mass is 1020 km distant at Cape Hatteras, USA (Gable et al. 2010). Given the long dispersal distance, we sought to understand how each of the talitrid taxa dispersed from the American continent and colonized Bermuda within the last 0.4 MYA. Three dispersal mechanisms were considered as hypotheses in the colonization process:

1. Synanthropic: as a passenger in some commodity of human commerce, such as the export of tropical plants and their soil to new locations, e.g. the so-called 'tramp' terrestrial talitrids (Birnbaum 1980; Morino and Ortal 1993; Stock and Birnbaum 1994).
2. Driftwood: as a driftwood talitrid population, which is a passenger on a floating driftwood log (Wildish 2012).
3. *Sargassum* wrack: as a passenger on a floating raft of *Sargassum* wrack.

The American continent and the proximate archipelagos of the Bahamas and Antilles have many wrack generalist talitrids (Ciavatti 1989; Wildish and LeCroy 2014). In addition, the Florida and Antilles currents, which merge into the Gulf Stream (Anon 1979), bathe its continental mainland and islands, carrying floating objects from a southwesterly origin towards Bermuda. In summer the winds are predominantly westerlies (Anon 1979), so reinforcing the drift towards Bermuda (Figure 1). Recent oceanographic modelling shows a clockwise movement around Bermuda (Ardron et al. 2011), which is a general feature of circulation in the Sargasso Sea.

Considering wrack generalist talitrids, an untested hypothesis is that they are carried in wrack used to transport valuable cargo in ships, such as live bivalves. As far as we are aware there are only a few precedents for a wrack generalist to be transported by human commerce. One is *Orchestia gammarellus* (Pallas 1766) from Europe to North America (Henzler and Ingolfsson 2008). The evidence here was circumstantial: the similarities in mitochondrial DNA between modern populations in Europe and North America. Another is *Platorchestia platensis* (Krøyer 1845) (Griffiths et al., 2009), although here the evidence for synanthropic dispersal is simply the wide distribution of this taxon throughout the world from its presumed native home on the Atlantic coast of the Americas. Because of the

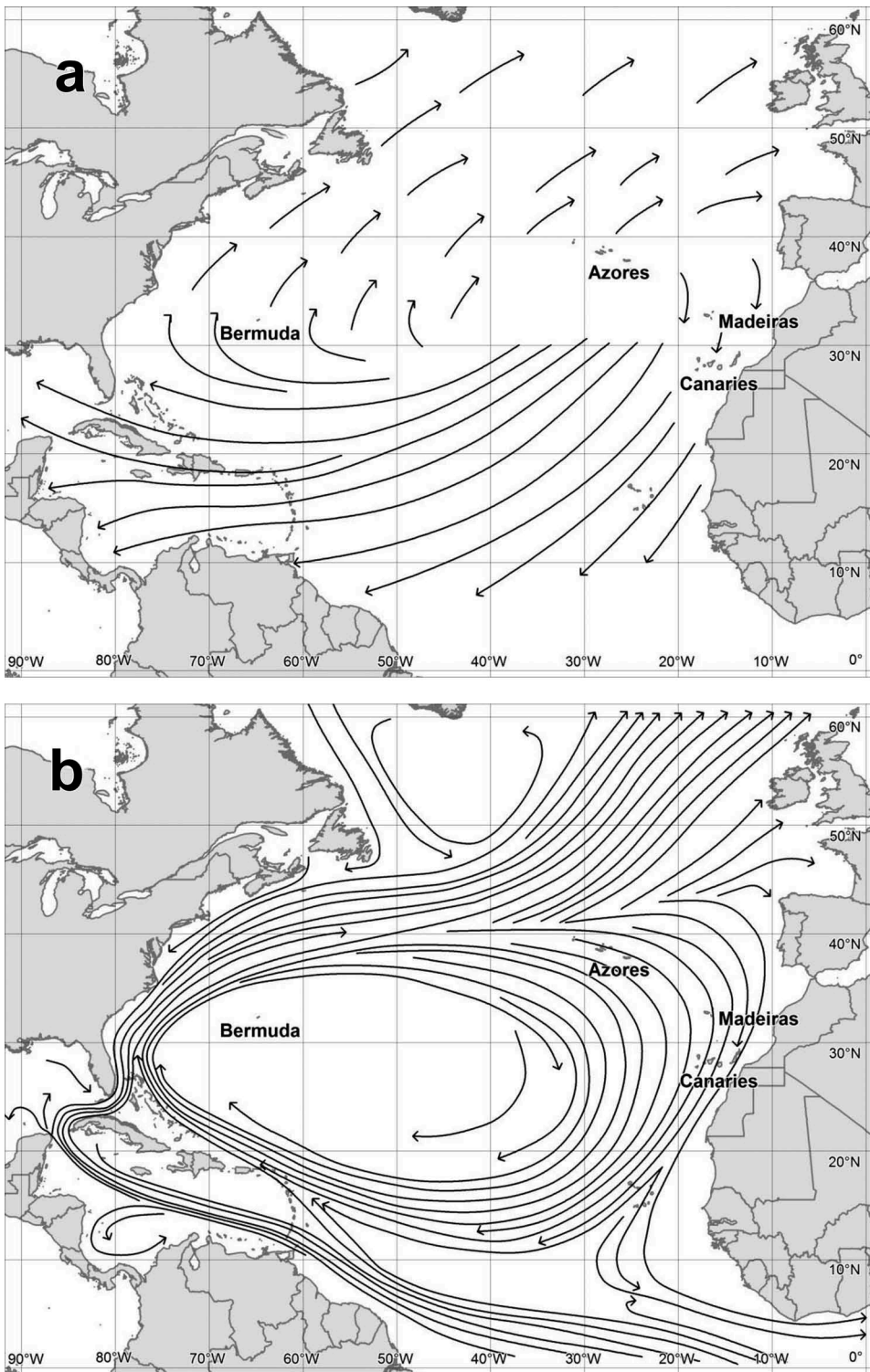


Figure 1. Map of the North Atlantic. (a) Winds in July. (b) Surface currents. Based on data in Anon (1979).

relatively recent human occupation of Bermuda, the talitrid genetic signature would be very similar to that on the continental mainland if synanthropic dispersal had been involved. Another plausible hypothesis is that supralittoral wrack generalists are transported on *Sargassum* wrack, after it strands on beaches in the Antilles, Bahamas or Florida, where it picks up travelling talitrids, is then blown out to sea, and follows currents and wind towards Bermuda. The special reason why *Sargassum* wrack may be an agent of long-distance dispersal is that the two major species in the Sargasso Sea, *Sargassum natans* (L.) (Gaillon 1828) and *Sargassum fluitans* (Børgesen) (Børgesen 1914) are holopelagic and achieve a pelagic mode of life with air-filled pneumatocysts (Lobban 1994). It is this flotation capability of *Sargassum* species that may allow rafts of it to persist long enough to transport wrack generalist talitrids for distances of up to 1500 km from North America, and its near-shore islands, to Bermuda. However, none of the reports that identified the macrofauna associated with floating *Sargassum*, e.g. Stoner and Greening (1984), have found talitrids to be present. Other wrack types are infrequently found on Bermuda's beaches, including *Fucus* sp., *Ascophyllum* sp., and *Spartina* sp. (S.R. Smith pers. observ.), which suggests connections with coastlines of temperate and subtropical Atlantic North America. A final possibility, applicable particularly to *Platorchestia* sp., because it is small enough to use gribble burrows on aged driftwood (Wildish and Robinson, in preparation) and thereby hitch a ride on driftwood, is that it reached the island by this means. These studies have shown that *Platorchestia platensis* is capable of living permanently in driftwood. In their review Thiel and Haye (2006) summarize reports of episodic natural rafting events in the northwest Atlantic following intense hurricane or storm events, which cause terrestrial debris, inclusive of driftwood, to be found floating at the sea surface. The timing between such events is unknown, although Thiel and Haye (2006) speculate that many years or even centuries between driftwood dispersal events could be involved.

A talitrid hopper was first described from Bermuda shores by Kunkel (1910) as *Orchestia platensis* (Krøyer 1845). The most recent amphipod list (Gable et al. 2010) also includes this species, now referred to as *P. platensis* (Krøyer 1845). Hence, in published accounts of the supralittoral talitrid fauna of the island, only a single taxon was recorded. In passing, Shoemaker (1935) mentioned the presence of a terrestrial species (as *Talitrus alluaudi* Chevreux 1896) in a shipment of celery grown in Bermuda which had been transported to, and found in, New York City.

Our aim was to present the results of field sampling designed to update the talitrid biodiversity of the supralittoral habitats on the island archipelago of Bermuda. We used modern molecular methods as a means of studying the dispersal history of the wrack generalist talitrids of Bermuda. Also of interest was the possibility that talitrids dispersed to Bermuda as driftwood specialists, as suggested by Wildish (2012) for talitrids from the northeast Atlantic mainland to the Azores.

Methods

Field sampling

Nineteen sampling locations (Figure 2, Table 1) were chosen where wrack and driftwood were likely to strand in the supralittoral zone, i.e. in sheltered bays and on sand beaches. Hand sampling was facilitated by shaking wrack or sand in a large plastic bag and

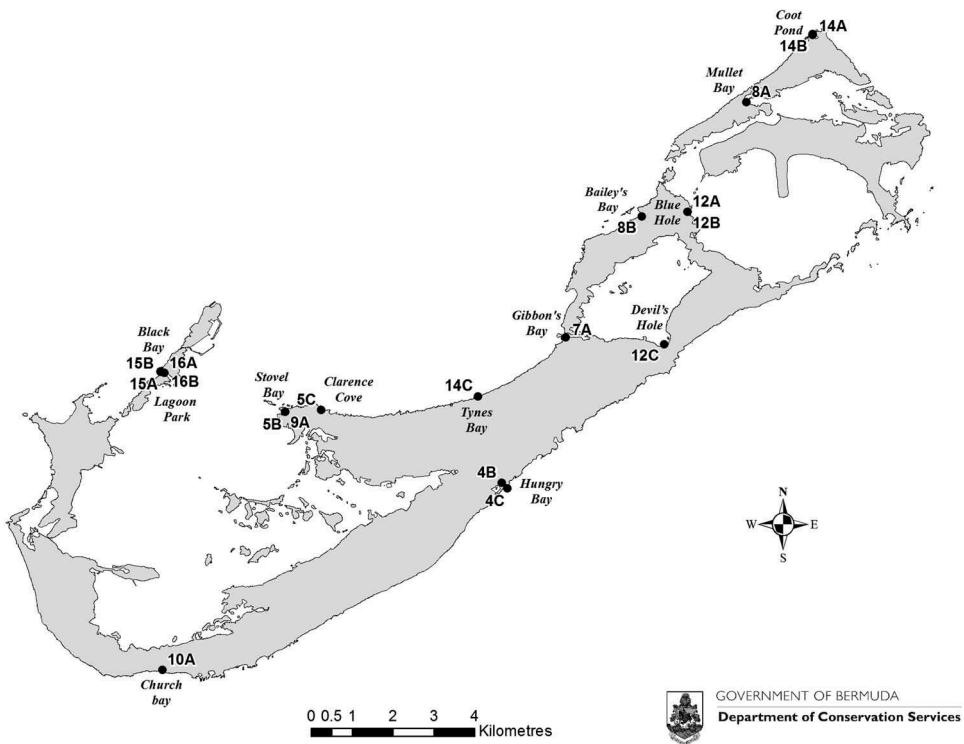


Figure 2. Map of the Bermudian archipelago showing sampling locations.

allowing the hoppers to jump into the bag. Driftwood was sampled by cutting the wood open with a small hand axe. Where holes were present in the sand it was sampled by hand digging into the substratum. Hand sampling between finger and thumb was also used. Where shrubs and terrestrial leaf litter occurred immediately above the storm wrack zone, a search was made in the humus layer for terrestrial hoppers, but this was not extended further inland, so the complete distribution of landhoppers on the island was not investigated. At each location sampled a habitat photograph was taken with a Canon D10 (Canon Inc, Japan) and a position fix was recorded with a 12-channel GPS (Garmin International, Inc., KS, USA).

Live animals were transported in plastic bags with a little dampened wrack or sand from the habitat to the Bermuda Natural History Museum laboratories. Killing and preservation of the catch was performed either in 5% formalin in local seawater (for morphological taxonomic analysis) or in 95% ethanol (for molecular analysis). Initial sorting of the samples was made with the aid of a binocular microscope. Taxonomic analysis based on the key of LeCroy (2011) and references therein was completed in the histology laboratory of the St Andrews Biological Station with the aid of a Leica MZ 12.5 binocular and Zeiss monocular microscope. Photographs of the dorsal pigment patterns (DPPs) were taken with a Canon CoolPix 990 digital camera mounted on the binocular scope. DPP diagrams were prepared by the fully digital method as in Wildish and Martell (2013). Temporary slides were prepared after dissecting body parts and were photographed to produce digital drawings as for the DPP diagrams.

Table 1. Supralittoral sampling locations in Bermuda.

Location number	Name	Coordinates		Substrate type	Ethanol <i>n</i>	BIN (<i>n</i>)	Formalin <i>n</i>	Genus & sp.	BAMZ Accession number
		North	West						
4A	Hungry Bay	32 17.439	64 45.518	T			1	<i>Talitroides alluaudi</i>	2016 295 001
4B	Hungry Bay	32 17.363	64 45.529	S, D			85	<i>Platorchestia</i> sp.	2016 295 002
4C	Hungry Bay	32 18.045	64 44.087	S			15	<i>Platorchestia</i> sp.	2016 295 003
5A	Spanish Point	32 18.379	64 48.897	T			30	<i>Talitroides alluaudi</i>	2016 295 004
5B	Stovell's Bay	32 18.379	64 48.897	S	3	AAC1491(1), AAB3402(2)	13	<i>Mexorchestia carpenteri carpenteri</i>	2016 295 005
5C	Clarence Cove	32 18.401	64 48.332	S	3	AAB3402(3)	20	<i>Platorchestia</i> sp.	2016 295 006
7A	Gibbon's Bay	32 19.359	64 44.516	S, D	4	AAB3402(3)	21	<i>Platorchestia</i> sp.	2016 295 007
8A	Mullet Bay			R	1				
8B	Bailey's Bay			D	1	AAB3402(1)	1	<i>Platorchestia</i> sp.	2016 295 008
8C	Bailey's Bay			S, D	3	AAA2949(3)	15		
9A	Stovell's Bay	32 18.379	64 48.897	S	4	AAC1491(4)	22	<i>Platorchestia</i> sp. & <i>Mexorchestia carpenteri carpenteri</i>	2016 295 009 & 2016 295 010
10A	Church Bay	32 14.958	64 50.805	S			12	<i>Platorchestia</i> sp.	2016 295 011
11A	Spanish Point	32 18.379	64 48.897	T	14	ACH9326(14)	32	<i>Platorchestia</i> sp. & <i>Talitroides alluaudi</i>	2016 295 012 & 2016 295 013
12A	Blue Hole	32 21.025	64 42.618	S, D	3	AAB3402(3)	13	<i>Platorchestia</i> sp.	2016 295 014
12B	Blue Hole	32 21.025	64 42.618	R					
12C	Devil's Hole	32 19.268	64 42.978	R			1	<i>Platorchestia</i> sp.	2016 295 015
14A	Tobacco Bay Park	32 23.381	64 40.663	R, D	3	AAA2949(1), AAB3402(2)	50	<i>Platorchestia</i> sp.	2016 295 016
14B	Coot Pond	32 23.366	64 40.659	S	1		12	<i>Tethorchestia antillensis</i> & <i>Platorchestia</i> sp.	2016 295 017 & 2016 295 018
14C	Tynes Bay	32 18.586	64 45.887	S	3	AAA2949(3)	26	<i>Platorchestia</i> sp.	2016 295 019
15A	Black Bay	32 18.905	64 50.839	S	3	AAB3402(3)	50	<i>Platorchestia</i> sp.	2016 295 020
15B	The Lagoon	32 18.908	64 50.835	S, R					
16A	Lagoon Park	32 18.889	64 50.779	R, D					
16B	Lagoon Park	32 18.889	64 50.779	R, D	4	AAA2949(3), AAB3402(1)	4		

Location number indicates sampling day in April 2014. Substrates: S, sand; R, rocky; D, driftwood; T, terrestrial leaf litter. Formalin *n* is the number of talitrids collected. Identifications denoted generically by the BIN (Barcode Index Number). Formalin-preserved specimens transferred to 50% isopropanol and archived at the Bermuda Aquarium, Museum and Zoo (BAMZ). Ethanol *n* is the number of preserved specimens archived at the Biodiversity Institute of Ontario, University of Guelph.

Generating novel barcode sequences

Five morphologically defined species were found to be present in Bermuda (Table 1). In addition to the Bermuda samples, we also analysed two other major regions as potential source populations: Florida and Mississippi in the Gulf of Mexico, and the Atlantic coast from New Brunswick to South Carolina.

Molecular analyses were carried out at the Canadian Centre for DNA Barcoding at the Biodiversity Institute of Ontario, University of Guelph. All 80 specimens selected for molecular analysis (1–14 individuals per location depending on availability) and stored in 95% ethanol were photographed, and a 2–4 mm length of tissue was removed for molecular analysis. In addition to the Bermuda samples (53 specimens), other available individuals from the Atlantic coast of North America (27 specimens) were also included in the molecular analysis.

Total genomic DNA was extracted from small amounts of tissue (2–4 mm) using one or two legs or the entire specimen when necessary with the glass fibre plate DNA extraction protocol (Ivanova et al. 2006). The barcode region of cytochrome *c* oxidase subunit I (658 base pairs; COI-5P) was amplified using a primer cocktail termed C_LepFolF (LepF1:LCO1490) – C_LepFolR (LepR1:HCO2198) (Folmer et al. 1994; Hebert et al. 2004) and following standard polymerase chain reaction protocols (Ivanova et al. 2006). The barcode region was bi-directionally sequenced using the same primers. Additional details about laboratory protocols are available from the Canadian Centre for DNA Barcoding website (www.dnabarcoding.ca).

All photographs, edited sequences and chromatograms were uploaded to the Barcode of Life Data Systems (BOLD; www.boldsystems.org, Ratnasingham and Hebert 2007) under the title ‘Talitridae of the Atlantic’ project (TLQMA), along with detailed information about each specimen. The project TLQMA includes samples from Bermuda and Florida (this project is publicly available and sequences have been transferred to GeneBank).

Molecular data analysis

In addition to successful new sequences from project TLQMA, 71 additional publicly available sequences (Radulovici 2012) from *P. platensis*, *Mexorchestia carpenleri carpenleri* (Wildish and LeCroy, 2014) and *Mexorchestia carpenleri raduloviciae* (Wildish and LeCroy, 2014) from our geographic area of interest were downloaded from BOLD from projects WWTAL (‘Barcoding Amphipoda – Talitridae’) and WWGSL (‘Crustaceans of the St Lawrence Gulf’) and combined with our novel data set to create the analysis data set DS-TLQPLATO, with 134 COI-5P sequences to be used in the following analysis. This dataset includes samples from the other two major geographical regions (Florida and Mississippi, and South Carolina and New Brunswick) that could be potential source populations. GenBank accession numbers, BOLD IDs, and all details about DS-TLQPLATO can be found at: <http://dx.doi.org/10.5883/DS-TLQPLATO>.

DNA sequences from the DS-TLQPLATO data set were aligned in MEGA version 6 (Tamura et al. 2013). The alignment was visually checked for gaps and translated to amino acids to verify the reading frame and absence of stop codons. The alignment was trimmed to 657 base pairs.

The best-fit molecular evolutionary substitution model was obtained in MEGA6. According to the Bayesian Information Criterion (BIC), where the lowest BIC score is considered the one that describes the substitution pattern best without model overfitting, the Tamura three-parameter model (Tamura 1992) with γ -distribution and invariant sites parameters (T92 + G + I) was the best model describing our data set and so was used in the following analysis. Phylogenetic relationships were reconstructed in MEGA-6 using the maximum likelihood method based on the T92 + G + I model and using two sequences of the genus *Americorchestia* as the outgroup. *Americorchestia megalophthalma* (Bate 1862) and *Americorchestia longicornis* (Say 1818) were chosen as outgroups, based on phylogenetic relationships from a maximum likelihood tree built using all publicly available sequences of the family Talitridae available in BOLD. Sequences of *A. megalophthalma* (sample ID: L230AR1-02) and *A. longicornis* (sample ID: L169AR1-01) were publicly available for downloading from BOLD (Radulovici et al. 2009).

The geographic distribution of genetic diversity was explored through haplotype networks. As marine morphologically defined species are frequently found to contain cryptic evolutionarily distinct species (Knowlton 1993; Bucklin et al. 2011; Carr et al. 2011), sequences were first grouped into Molecular Operational Taxonomic Units according to the Barcode Index Number (BIN) algorithm (Ratnasingham and Hebert 2013). BIN assignments are available through BOLD for all COI sequences with length > 500 base pairs and meeting quality standards (that is within a specific region, and having fewer than 1% Ns). Haplotype networks were created for three BINs: BOLD:AAB3402 (*Platorchestia monodi* Mateus, Mateus and Afonso, 1986), BOLD:AA2949 (*P. platensis*), and BOLD:AAC1491 (*M. c. carpenteri*). For each BIN, a median-joining haplotype network (Bandelt et al. 1999) was created in PopArt (PopART: www.popart.otago.ac.nz). Haplotype networks were then recreated in TCS version 1.21 with a 95% connection limit and taking into consideration missing data from both ends of the alignment, in order to identify ancestral haplotypes (Clement et al. 2000, 2002).

Population genetics analyses were performed only for BIN BOLD:AAB3402, as that was the only BIN with a sample size of > 30 individuals. All sequences from BIN BOLD:AAB3402 residing within data set DS-TLQPLATO were analysed, for a total of 23 sequences from Bermuda and 34 sequences from Florida. Alignment and phylogenetic analysis of BIN BOLD:AAB3402 were performed in MEGA-6. According to the BIC criterion, the best substitution model for this set of sequences was the Tamura three-parameter model (T92). Arlequin 3.5 (Excoffier and Lischer 2010) was employed to identify haplotypes and to examine molecular diversity indices and patterns of genetic structure in the BIN. To determine how genetic variance was distributed within and between populations, as a means to test for between-population differentiation (Excoffier and Lischer 2010), an analysis of molecular variance (AMOVA) was conducted using the conventional F-statistics with 1000 permutations.

To determine the partitioning of genetic variance (Weir and Cockerham 1984) the Fixation index (F_{ST}) was also estimated considering the average number of pairwise differences between haplotypes (π), 1000 permutations, and with a significance level of 0.05. An F_{ST} value of 0 indicates that subpopulations have the same allele frequencies, and there is no genetic differentiation among subpopulations. On the other hand, an F_{ST} value of 1 indicates strong genetic differentiation among subpopulations as they do

not share alleles (Hedrick 2005). Additionally a Tajima's *D*-test was performed in Arlequin 3.5 to test for deviations from neutral evolution in the barcode sequences.

Results

Systematics and distribution of morphological species

Platorchestia monodi (Mateus, Mateus and Afonso, 1986) BOLD:AAB3402

As *Orchestia platensis* Krøyer 1845, p. 304, fig. 2; *Platorchestia platensis* Krøyer 1845, Bousfield 1982, p. 26; *Orchestia monodi* Mateus, Mateus and Afonso, 1986, p. 100–110, figs 1–7; *Platorchestia monodi* Morino and Ortal 1993, p. 825–829, figs 1–3; LeCroy 2011, p. 754–757, fig. 597.

Material examined

From the following sampling locations: 4B, 4C, 5C, 7A, 8B, 9A, 10A, 11A, 12A, 12C, 14A, 14B, 15A (see Table 1 and Figure 2 for further details). Note that 9A is the same sample location as 5C, but made at a later date.

Distribution

Found on most suitable beaches throughout the islands where wrack can strand. Besides its primary ecotope, under and in wrack, some specimens were found at 4C (Hungry Bay) within the sand substratum, limited to a few centimetres depth (see also Bousfield 1984, who records *P. platensis* from a similar habitat). Circular holes of 5–8 mm diameter near the *P. monodi* ecotope were thought not be associated with the animals, but to result from physical effects after high tide. Such holes, lacking any evidence of digging wastes, were found on other sand substrata where talitrids were absent. Two other secondary ecotopes identified were: coastal terrestrial soil litter (two female individuals) associated with *T. alluaudi* (11A), and in driftwood (see below).

Remarks

Present in 13 of 19 sampling locations and the commonest wrack generalist taxon. Dorsal pigment patterns are obscured by background colours and are not useful for field identification.

Platorchestia platensis (Krøyer, 1845) BOLD:AAA2949

As *Orchestia platensis* Krøyer 1845, p. 304, fig. 2; *Platorchestia platensis* (Krøyer 1845), Bousfield 1982, p. 26.

Material examined

From the following sampling locations: 8C, 14A, 14C, 16B (see Table 1 and Figure 2 for further details).

Distribution

Found on similar sand beaches and under similar wrack piles as *P. monodi*. Found in single species samples (that is all three individuals analysed = BOLD:AAA2949), all on the northwest coast of Bermuda at Bailey's Bay (8C), Tynes Bay (14C) and Lagoon Park (16B). One other sample at Tobacco Bay Park (14A) was a mixed sample containing one *P. platensis* and two *P. monodi*. Other locations on the northwest coast consisted of single species *P. monodi*, e.g. Coot Pond (14B), Gibbon's Bay (7A) and Stovell's Bay (5B).

Remarks

During this study this species was first recognized by its distinct molecular signature, which matched specimens identified as *P. platensis* from BOLD and GenBank. The same species is common on the Atlantic coast of North America, north of Charleston, North Carolina to New Brunswick (Radulovici 2012). Formalin-preserved specimens have not been checked for the possible occurrence of the two species of *Platorchestia* by morphological criteria, with the exception of sample 8C.

***Mexorchestia carpenteri carpenteri* Wildish and LeCroy, 2014 BOLD: AAC1491**

As *Tethorchestia* sp. B, Bousfield 1984, p. 204; '*Tethorchestia*' LeCroy 2011, p. 757; *Mexorchestia carpenteri carpenteri* Wildish and LeCroy 2014, p. 562.

Material examined

Two samples, 5B and 9A, $n = 14$, both from Stovell's Bay on Spanish Point. This taxon was found in the same bleached *Enteromorpha* wrack with *P. monodi* BOLD: AAB3402.

Distribution

This taxon was found in only one location, Stovell's Bay, of the 19 locations sampled.

Remarks

Live specimens can readily be distinguished by the conspicuous red and black epidermal pigment patterns present on a pale white or fawn-coloured background (Figure 3). In the Florida specimens of this taxon (Wildish and LeCroy 2014) the pigment patterns are also variable. It is of interest that the bauplan of the Bermuda dorsal pigment patterns are very similar to those recorded by Wildish and LeCroy (2014, see fig. 6D); for coastal Atlantic specimens of this taxon at Fort Lauderdale, Florida.

***Tethorchestia antillensis* Bousfield 1984**

Tethorchestia antillensis, Bousfield 1984, p. 205; Ciavatti 1989, p. 139; LeCroy 2011, p. 759; Wildish and LeCroy 2014, p. 558.

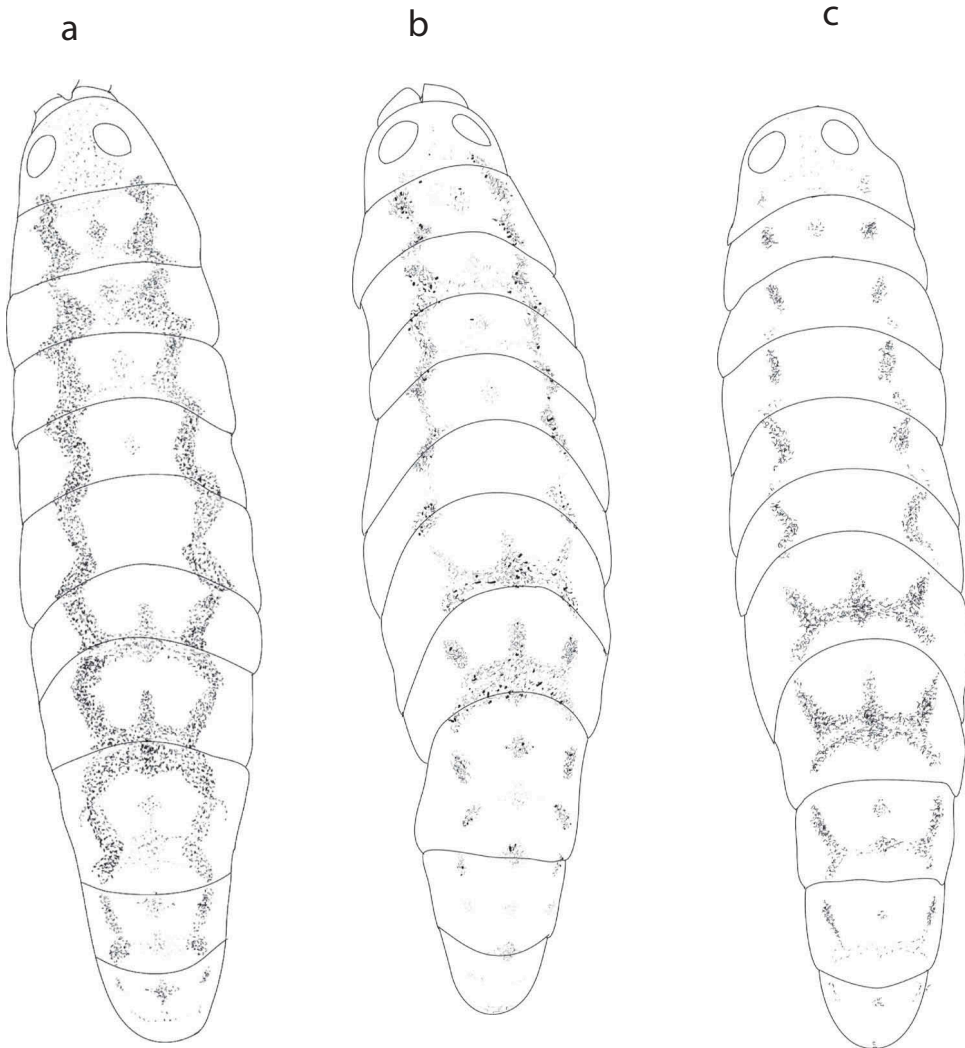


Figure 3. Dorsal pigment pattern diagrams of three individuals (a, b and c) from a single population of *Mexorchestia carpenteri carpenteri* from Stovell's Bay sampled on 5 or 9 April, 2014.

Material examined

A single sample, 14B, from Coot Pond, $n = 8$.

Distribution

This taxon was found in only one location, at Coot Pond, co-habiting the same wrack as *P. monodi* BOLD: AAB3402. Coot Pond is a small inland bay with restricted tidal access, so the tidal amplitude may be slightly reduced.

Remarks

This is the largest of the Bermudian talitrids, up to 17 mm in body length. Epidermal pigment patterns are nondescript and not useful for field identification.

***Talitroides alluaudi* (Chevreux, 1896) BOLD:ACH9326**

As *Talitrus alluaudi* Chevreux 1896, p 112; *Talitroides alluaudi* Chevreux, 1896, Palmén 1949, p 61; Morino and Ortal 1993, p 332.

Material examined

4A, Hungry Bay mangrove forest in leaf litter; 5A, Spanish Point on limestone cliff, under terrestrial shrub leaf litter; 11A, near the same location as 5A.

Distribution

Only coastal locations incidental to the island-wide, supralittoral survey were sampled, so we are unable to describe the general distribution of this taxon in terrestrial habitats of Bermuda.

Remarks

The smallest talitrid at < 7 mm body length. This terrestrial taxon is probably common throughout the island where damp, well-rotted humus is found under leaf litter.

Key for the talitrids of Bermuda

1. Immature and adults < 7 mm body length, second antenna < 25% of body length *Talitroides alluaudi*
 Immature and adults > 7 mm body length, second antenna > 25% of body length **2**
2. Male second gnathopod dactylus with blunt tip and incised propodus palm; second antenna peduncle much enlarged in older males..... **3**
 Male second gnathopod dactylus with fine tip and smooth propodus palm; second antenna peduncle not, or only slightly, enlarged in male..... **4**
3. Male adult pereopod 7 merus and carpus not incrassate; ovigerous female in oostegite 5 with equal numbers of fine setae on anterior and posterior margins *Platorchestia monodi*
 Male adult pereopod 7 merus and carpus incrassate; ovigerous female with more fine setae on the anterior than the posterior margin of oostegite 5.....
 *Platorchestia platensis*
4. Dorsal pigment patterns on anterior pereopod absent; distolateral robust seta present on first uropod peduncle; four to seven tufts of 'comb' setae on male propodus of pereopod 7..... *Tethorchestia antillensis*
 Dorsal pigment patterns conspicuous (Figure 2); distolateral robust seta absent on first uropod peduncle; maximum of four tufts of 'comb' setae on male propodus of pereopod 7..... *Mexorchestia carpenteri carpenteri*

N.B. The few distinguishing features between the two *Platorchestia* species are based on LeCroy (2011), and this reference should be consulted. Only adult males and females can be identified morphologically.

The formalin-preserved specimens, now in 50% isopropanol, are archived at the Bermuda Aquarium, Museum and Zoo (BAMZ) at Flatt's Village, Bermuda, with the exception of two samples (8C archived at the Atlantic Reference Centre, Huntsman Marine Science Centre, St Andrews, N.B., Canada, and 16B formalin sample lost during sorting). Ethanol-preserved specimens, with the exception of those few consumptively analysed for DNA extraction, are archived at the Biodiversity Institute of Ontario, University of Guelph, Ont., Canada

Natural history

One feature of the Bermuda coastline is the relatively few beaches available where wrack and driftwood can strand. The popular South-shore beaches are cleaned of wrack in the summer months for the benefit of tourists. In winter months wrack removal is less frequent and, as pointed out by Healy and Coates (2003), this provides an opportunity for animals, including talitrids, to become established. Most of the coastline consists of a vertical, limestone, eroding cliff-face from a few to 30 m in height above sea level, where seaborne drifting materials are unable to strand. Nineteen beach locations were investigated, most < 50 m in linear length, which were spread throughout Bermuda (Figure 2). The name and co-ordinates of each sampling location are further specified in Table 1.

The following marine and terrestrial ecotopes harbouring talitrids were recognized in Bermuda:

1. Supralittoral, semi-tropical wrack on sand (Table 1). The wrack consisted principally of *Sargassum* sp., sometimes mixed with other macroalgae, such as *Enteromorpha* sp., and sometimes with leaves of native terrestrial shrubs, such as the button-wood, *Conocarpus erectus* L., and the bay grape, *Coccolobo* sp. At location 5B (Figure 2 and Table 1), which contained both *P. monodi* and *M. c. carpenteri*, the wrack was principally of senescent, bleached *Enteromorpha* sp., with *Sargassum* sp., also present. The wrack at Coot Pond (14B in a small inland sea), with both *P. monodi* and *Tethorchestia antillensis*, consisted mostly of leaves of land plants with a little *Sargassum* sp. The upper littoral of the inland sea at Coot Pond was colonized by unidentified woody shrubs. From a total of 19 supralittoral locations sampled, 13 were mostly of limestone sand. At Lagoon Park (16B) a small bivalve midden (valves of *Arca zebra*) with buried *Sargassum* and terrestrial plant leaves, a few *P. platensis* BOLD:AAA2949 and *P. monodi* BOLD:AAB3402 were found.
2. Supralittoral, semi-tropical wrack on a rocky shore (Table 1). The shore at Mullet Bay (8A) consisted of small limestone rocks with very little wrack. At Blue Hole (12B), the station sampled was close to the road causeway and was of mixed sand and small limestone rocks with small amounts of *Sargassum* wrack, making sampling difficult. At Devil's Hole (12C), in the inland lagoon of Harrington Sound, the rocky shore was abbreviated by a wall with the wrack being of rubbish and terrestrial plant leaves. At Tobacco Bay Park (14A) on the seaward side of the coastline, copious *Sargassum* wrack mixed with anthropogenic litter was present on a rocky shore. At the Lagoon (15B), a small inland bay with

seawater exchange in a pipe passing under the road, a mixed sand/rocky shore had only terrestrial plant wrack. At Lagoon Park (16A) the upper shore consisted of large limestone rocks on which wrack and driftwood were drying, with no talitrids found. A total of six of the locations sampled had a rocky or mixed sand/rock substratum, and *P. monodi* BOLD:AAB3402 was found in Devil's Hole (12C) and a mixed sample at Tobacco Bay Park (14A), as shown in Table 1.

3. Supralittoral, semi-tropical driftwood (Table 1). No large tree trunks or whole trees were found during the fieldwork. The largest piece (~ 5 m length) was a branch at 4B with no evidence of talitrids, or other inhabitants, with hard consistency and no evidence of rotting. On the sandy shore at 4B mostly occupied by *Sargassum* wrack, two coconut husks were opened, with no talitrids present. A small log (~ 1 m long) was found at 7A without talitrids. At Bailey's Bay (8B) a small piece of driftwood (~ 30 × 15 × 5 cm) had obviously been at sea for an extended period as evidenced by the large holes (Figure 4) cut by the marine boring bivalve *Teredo* sp. A small wood sample from this was identified by Dr Peter Gasson of the Royal Botanic Gardens at Kew, UK, as spruce (*Picea* sp.) and hence not from Bermuda. Two slow-moving talitrids were retrieved from this sample and proved to be *P. monodi* BOLD:AAB3402. It is of interest that the talitrids collected from wrack on the sand beach at Bailey's Bay (8C) were *P. platensis* BOLD:AAA2949. Small log samples were found at 12A (45 cm long), 14A (30 cm), and 16A (both 50 cm long), all of which lacked talitrids. At 16A and 16B a coconut husk at each was opened



Figure 4. Driftwood photograph, consisting of spruce (*Picea* sp.) stranded in the wrack zone at Bailey's Bay on 8 April, 2014. Two individuals of *Platorchestia monodi* BOLD:AAB3402 were found within the *Teredo* burrows.

without finding talitrids. Similarly, a coconut with decomposing milk still present at 16B also did not contain talitrids. Hence, we found driftwood samples at only seven of the 19 locations sampled, with talitrids in only one sample. The *P. monodi* BOLD:AAB3402 present were clearly in a secondary habitat (Figure 4), and no driftwood specialist talitrids were found.

4. Terrestrial, semi-tropical, coastal, forest leaf litter. The microhabitat in which *T. alluaudi* was found appeared to be superficially similar at each of the two locations (Hungry Bay mangrove forest in leaf litter and Stovell's Bay on Spanish Point under shrubs on the limestone cliff) in which they were found, i.e. under terrestrial forest leaf litter where there was a dark-coloured, almost black humus layer. They were present with typical soil inhabitants, such as insect larvae, centipedes, millipedes, isopods, ants and also two individuals of *P. monodi* BOLD:AAB3402.

Molecular data

From the 80 specimens selected for generating novel sequences for this study, only 63 talitrid specimens were successfully amplified. The failures included nine specimens of *Platorchestia* sp. and all eight specimens attempted for *Tethorchestia antillensis*; therefore, we were only able to recover molecular data from four of the five morphologically defined species that were found in Bermuda. The strong taxon-specific pattern of polymerase chain reaction failure for *Tethorchestia antillensis* suggests substitution in COI in one of the primer binding regions, which requires further study. Most of the COI sequences included the full length of the barcode region (658 base pairs), but a few were slightly shorter.

DNA barcoding of talitrids of Bermuda showed four BINs, corresponding to four morphological species. Specimens from *M. c. carpenteri* (BOLD:AAC1491), *T. alluaudi* (BOLD:ACH9326), *P. monodi* (BOLD:AAB3402) and *P. platensis* (BOLD:AAA2949) were shown to be each monophyletic with other members of their species in a maximum likelihood tree (Figure 5). The clade of *P. monodi* was formed by individuals from Bermuda and Florida, whereas the clade of *P. platensis* contained specimens from both Bermuda and the North Atlantic coast.

Haplotype diversity

Haplotype networks were created to explore the haplotype distribution for three BINs found in Bermuda and other coastline localities from North America/Gulf of Mexico.

Seven haplotypes were identified for *P. monodi* BIN BOLD:AAB3402 among the 57 individuals sequenced. Three haplotypes are shared between the Florida and Bermuda localities. However, the other four haplotypes are only present in one of the two populations. One of the shared haplotypes is six or more mutational steps from all other haplotypes. Haplotype distribution by locality is presented in the median-joining network (Figure 6), where the ancestral haplotype according to the TCS analysis is marked with an asterisk. The median-joining network suggested that the presumptive ancestral haplotype is present only in Bermuda (Figure 6).

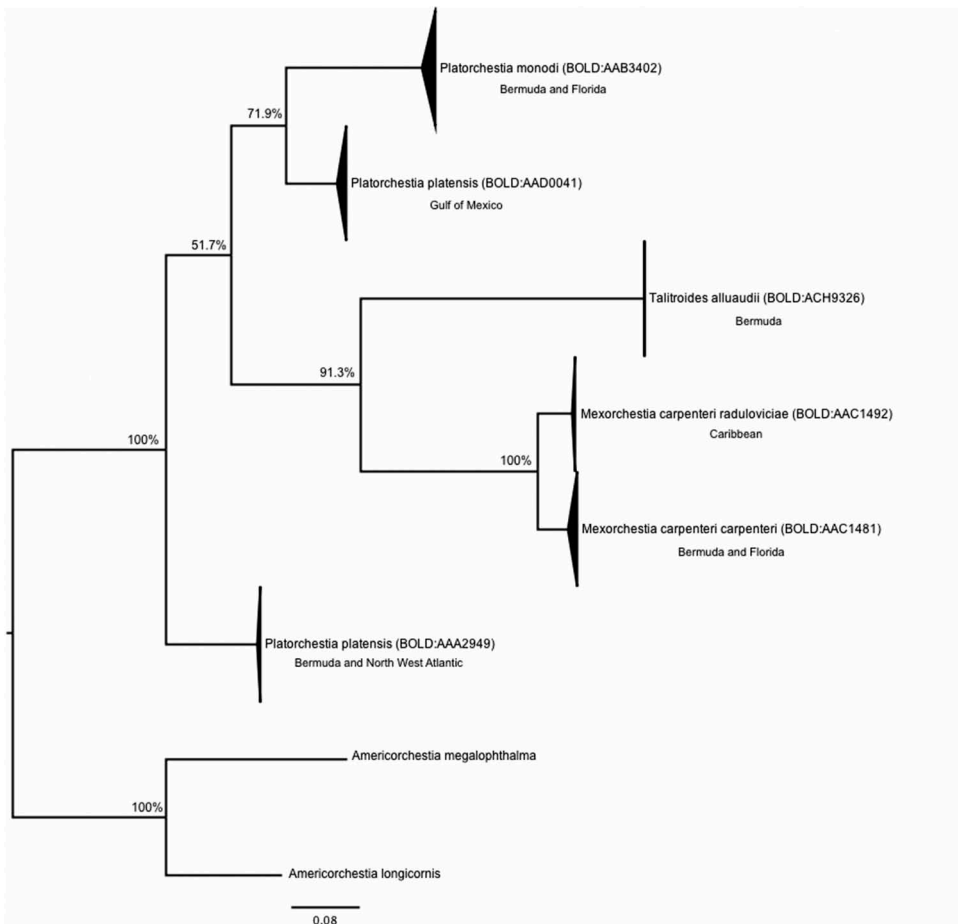


Figure 5. Maximum likelihood tree for DS.TLQPLATO data set. Molecular phylogenetic analysis of DS-TLQPLATO data set using the maximum likelihood method and based on the T92 + G + I model. The log likelihood value of the tree is -3244.0825 . Branch lengths are measured in the number of substitutions per site. Triangles represent compressed clades and bootstrap probabilities are shown on the tree. The origin of the samples is provided beneath the name of the tips.

For *P. platensis* BIN BOLD: AA2949, four haplotypes were identified among the 14 specimens assigned to this BIN (Figure 6). Only one haplotype, which has also been identified as the presumptive ancestral haplotype, is shared between Bermuda, South Carolina and New Brunswick localities. The other three haplotypes are unique for Bermuda or New Brunswick localities.

On the other hand, 11 haplotypes were identified for *M. c. carpenteri* (BOLD:AAC1491) from among the 21 specimens sequenced from this BIN (Figure 6). However, none of these haplotypes are shared between Florida and Bermuda localities. Two haplotypes are present in Bermuda, and are three or more mutational steps away from all Florida haplotypes, whereas the rest are only present in Florida. For this clade the presumptive ancestral haplotype occurs only in Florida, which is home to a population that is much more genetically diverse than that in Bermuda (Figure 6).

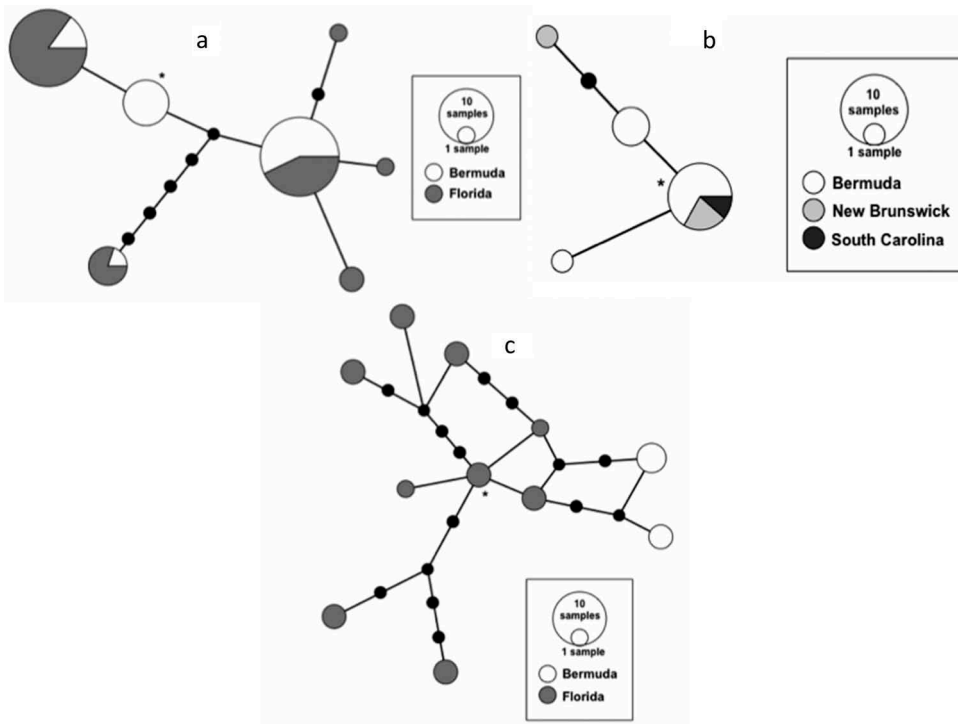


Figure 6. Haplotype network of partial cytochrome c oxidase subunit I sequences. (a) *Platorchestia monodi* BIN BOLD:AAB3402. (b) *Platorchestia platensis* BOLD:AAA2949. (c) *Mexorchestia carpenteri carpenteri* BOLD:AAC1491. Median-joining haplotype networks were created in PopART. All mutational steps are equal to 1 and are represented with a black dot. The presumptive ancestral haplotype is marked with an asterisk. The size of the circles varies according to the number of sequences belonging to each haplotype.

Population genetics analyses were not performed for BINs BOLD:AA2949 and BOLD:AAC1491, due to small sample size, but were performed for *P. monodi* BIN BOLD:AAB3402. The AMOVA suggests a significant degree of heterogeneity among populations with an $F_{ST} = 0.336$ ($p < 0.001$) (Table 2). Pairwise comparison between the two populations of *P. monodi*, when also considering haplotype distances, are consistent with the AMOVA results (corrected average pairwise difference = 0.3374, $p = 0.00000$). The results from AMOVA and pairwise comparison between populations revealed that most genetic variation was observed within (66.4%) rather than between (33.6%) popu-

Table 2. Analysis of molecular variance (AMOVA) among Bermuda and Florida populations of *Platorchestia monodi* BIN BOLD:AAB3402.

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation
Among populations	1	4.958	0.16857 Va	33.60
Within populations	55	18.322	0.33313 Vb	66.40
Total	56	23.281	0.5017	

Fixation index $F_{ST} = 0.336$
 $p = 0.00000+0.00000$

lations. The Tajima's D -tests for Bermuda (Tajima's $D = -0.87506$) and Florida (Tajima's $D = 0.10341$) populations were not significant (Bermuda $p = 0.195$, Florida $p = 0.605$), which is consistent with a neutral model of COI molecular evolution in this data set.

Discussion

Species diversity

As a result of this field survey the talitrid biodiversity of the island was increased from two to five taxa. Limited sampling and lack of taxonomic expertise in previous surveys undoubtedly played a role in this finding. Further sampling effort may increase talitrid biodiversity both for supralittoral and terrestrial species. Two salt marshes, at Cooper's Island and Spittal Pond, were not investigated, and so we were unable to determine if palustral talitrids were present on the Bermudian archipelago. No sand-burrowing specialists were found, despite the presence of suitable sand substrata in which to burrow. The likely reason is that dispersal events are much less common for sandhoppers (Wildish 2012) than for wrack generalists, although after violent wave events sand substrata can be completely removed, but perhaps without a supporting system of floating wrack or driftwood to which talitrids can cling. The primary ecotope of all of the supralittoral species found in Bermuda is wrack. For the commonest, *P. monodi* BOLD: AAB3402, three secondary ecotopes on the island – that is microhabitats to which the taxon is not primarily adapted – were recognized. These included: shallow sand burrowing, also reported in Bousfield (1982) for *P. platensis*, occupation of *Teredo* burrows in driftwood, and the terrestrial niche in plant litter humus, co-habiting with *T. alluaudi*.

Of interest are the two species of *Platorchestia* recognizable both by genetic differences (herein and in Radulovici 2012) and by morphological taxonomy at least in the oldest adults (LeCroy 2011). They exist on the island archipelago of Bermuda at the same locations, apparently without interbreeding.

Dispersal of talitrids to Bermuda

One goal of this research was to test the hypothesis that talitrids reached remote oceanic islands adapted as driftwood specialists transported in driftwood. Circumstantial evidence does support this mechanism in some other oceanic islands (Wildish 2012; Wildish et al. 2012; Pavesi et al. 2014). For Bermuda both field and genetic results support the null hypothesis that no specialized driftwood talitrids had reached the archipelago by transport on driftwood. We found few places where driftwood could strand and minimal driftwood. We cannot conclude that talitrid dispersal in driftwood did not occur in the past, because of the possibility of episodic natural rafting (Thiel and Haye 2006), either in *Sargassum* rafts or in driftwood. Episodic rafting has a time scale (years to centuries) which is much longer than our study period. Our results suggest, but cannot prove, that specialist driftwood talitrids, with characteristically dwarf body sizes, did not reach Bermuda in the immediate past. No specialist driftwood talitrids, with morphological dwarfism, have yet been identified anywhere on the American coastal margin. However, for smaller-sized talitrids, such as *Platorchestia* sp., dwarfism is unnecessary for driftwood

occupation, as they can live permanently in driftwood as a secondary ecotope (Wildish and Robinson, in preparation), and hence could have reached Bermuda as a driftwood passenger. We provide direct evidence that two individuals of *P. monodi* BOLD:AAB3402 were present in the same piece of stranded driftwood (Figure 4) and could have reached Bermuda by this means. It is also possible that they originated in Bermuda after multiple strandings of the driftwood specimen. O'Foighil and Josefowicz (1999) and O'Foighil et al. (2001), using molecular genetic inferences, have suggested that the brooding bivalve genus, *Lasaea*, reached Bermuda by some form of rafting dispersal from Florida.

Human settlement began in Bermuda in 1612 (Zuill 1999), with the arrival of colonists from England, although shipwreck survivors from earlier times were also present. This limits synanthropic dispersal of talitrids to the island to around 400 years ago. From the review of the geology of Bermuda in the Introduction, the island was available for colonization from ~ 400,000 years ago. For the only available dispersal mechanism of wrack generalist talitrids before human colonization of Bermuda, that is by episodic natural rafting, the establishment of new talitrid colonies in Bermuda can be dated by estimating the divergence times, based on mitochondrial DNA and the COI gene, between the North American mainland and island populations.

Our molecular results for *P. monodi* BOLD:AAB3402 show that the dominant haplotypes on Bermuda are six mutational steps (c. 0.9% sequence divergence) away from Florida samples and hence most likely originated from a dispersal event that occurred up to c. 0.6 MYA. This estimate is based on the assumption of a molecular clock of c. 1.4% sequence divergence per million years for the COI gene (Knowlton and Weigt 1998). Although there is probably a wide error margin associated with this estimate, it is approximately consistent with the geological estimate of 400,000 years ago, when the Bermudian supralittoral habitat would have been an empty one, and inconsistent with anthropogenic dispersal. For *P. monodi* BOLD:AAB3402, there was genetic evidence (Figure 6) that the Florida and Bermuda populations were not panmictic and that the presumptive ancestral haplotype is currently situated on Bermuda. Given the modest sample size of sequences, these results may be consistent with two different hypotheses: (1) that this species arose on the tropical/semi-tropical Atlantic coast of the continent of America and dispersed from there to the Atlantic islands, inclusive of Bermuda, the Azores, Madeira and Ascension. After dispersal to Bermuda the ancestral haplotype was either not found, or had become extinct, on the American continent. This species is known only by classical morphological taxonomic methods from the islands mentioned (excepting Bermuda). An alternative hypothesis (2) is: that this species arose on one of the Atlantic islands, and then dispersed to the continental mainland and other Atlantic oceanic islands. The shared haplotypes between mainland and island suggest multiple cases of dispersal, some perhaps less than 400 years ago, which could be due to synanthropic dispersal and so supplementing natural rafting to Bermuda.

For the other two talitrid populations analysed genetically, small sample sizes prevented further detailed population genetics analysis. The most plausible estimate for *P. platensis* BOLD:AAA2949 suggests a source population somewhere north of Florida (see Radulovici 2012), from where it dispersed in the Gulf Stream to Bermuda

before human habitation of the island; as well as to Europe (Pavesi et al. 2014). For *Mexorchestia* (BOLD: AAC1491), the haplotype network indicates genetic segregation between Bermuda and Florida, with the most diverse set of haplotypes in Florida, which indicates that the American continent is the source population. The two unique haplotypes on Bermuda are three mutational steps away from Floridean haplotypes, most likely indicating a pre-anthropogenic dispersal. This could possibly have been a single dispersal event and contrasts with the pattern in *P. monodi* BOLD: AAB3402, where multiple cases of haplotype sharing suggest multiple dispersal events. Judging by the predominance of *P. monodi* BOLD: AAB3402, it suggests that it is out-competing *P. platensis* BOLD: AAA2949 for the same wrack habitat on the island archipelago of Bermuda.

For the terrestrial talitrid *Talitroides alluaudi*, published accounts (Biernbaum 1980; Morino and Ortal 1993; Stock and Biernbaum 1994) all treat this as a 'tramp' talitrid, widely dispersed by synanthropic means. The only direct evidence that it is dispersed synanthropically of which we are aware is that of Shoemaker (1935), who found this species associated with commercial production of celery in Bermuda. During transport to New York, either in soil/leaf litter or among the fleshy stems of celery, the talitrids were able to survive. In the northern hemisphere it is widely dispersed in tropical plant hot houses, where imports of both plants and soil are known to have occurred.

All three of the dispersal mechanisms considered seem to be supported by one or other of the wrack generalist species considered here. Hence, synanthropic, driftwood, or wrack dispersal is possible for *P. monodi* BOLD: AAB3402; driftwood or wrack transport for *P. platensis* BOLD: AAA2949; and *Sargassum* rafts for *M. c. carpenteri*. However, before firm conclusions on talitrid dispersal mechanisms to Bermuda from the American mainland can be made, additional work is needed, including further field sampling and sequencing from the full geographic range of each species. The occurrence of two morphologically similar, congeneric species: *P. monodi* BOLD: AAB3402 and *P. platensis* BOLD: AAA2949 in sympatry in Bermuda is intriguing. Future studies to determine how interbreeding is prevented are clearly indicated. We found no ecological factors to suggest that each species occupied separate niches. In some cases they occupied the same location and hence both taxa were competing for the same habitat resources. Our results clearly demonstrate that long-distance dispersal (> 1000 km) has been achieved by four species of wrack generalist talitrids.

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References

- Anon. 1979. World Atlas. Montreal: Geografic International.
- Ardron J, Halpin P, Roberts P, Cleary J, Moffit M, Donnelly J. 2011. Where is the sargasso Sea? a report submitted to the Sargasso Sea alliance. Sargasso Sea Alliance Science Report Series No. 2. p. 24.
- Bandelt HJ, Forster P, Rohl A. 1999. Median-joining networks for inferring intraspecific phylogenies. *Mol Biol Evol.* 16:37–4.
- Bate CS. 1862. Catalogue of the specimens of amphipodus Crustacea in the collection of the British Museum. London: British Museum. p. 399.
- Biernbaum CK. 1980. Occurrence of the tramp terrestrial amphipods *Talitroides alluaudi* and *T. topitotum* in S. Carolina. *Brimleyana.* 3:107–111.
- Børgesen F. 1914. The marine algae of the Danish West Indies. Part 2. Phaeophyceae Dansk Botanisk Arkiv. 2:1–68.
- Bousfield EL. 1982. The amphipod superfamily Talitroidea in the northeastern Pacific region. 1. Family Talitridae: systematics and distributional ecology. Ottawa: Publications in Biological Oceanography, No.11; p. 73.
- Bousfield EL. 1984. Recent advances in the systematics and biogeography of the landhoppers (Amphipoda: talitridae) of the Indo-Pacific region. *Bishop Museum Spec Publ.* 72:171–210.
- Bucklin A, Steinke D, Blanco-Bercial L. 2011. DNA barcoding of marine metazoa. *Ann Rev Mar Sci.* 3:471–508.
- Carr CM, Hardy SM, Brown TM, Macdonald TA, Hebert PDN. 2011. A tri-oceanic perspective: DNA barcoding reveals geographic structure and cryptic diversity in Canadian polychaetes. *PLoS One.* 6: e22232.
- Chevreaux E. 1896. Recherches zoologiques dans les serres du Museum de Paris. IV. Sur un Amphipode terrestre exotique, *Talitrus Alluaudi* nov. sp., acclimaté dans les serres du Jardins des Plantes de Paris. *Feuille des Jeunes Naturalistes.* 26:112–113.
- Ciavatti G. 1989. Les talitres (Crustacea. Amphipoda) des plages de la Guadeloupe. Description de deux espèces nouvelles. *Ann Inst Oceanogr, Paris.* 65:127–146.
- Clement M, Posada D, Crandall KA. 2000. TCS: a computer program to estimate gene genealogies. *Mol Ecol.* cited 2015 Aug 10; 9. 1657–1659. doi:10.1046/j.1365-294x.2000.01020.x
- Clement M, Snell Q, Walke P, Posada D, Crandall K. 2002. TCS: estimating gene genealogies. Proceedings of the 16th International Parallel Distributed Process Symposium; 2001 April; Fort Lauderdale (FL).
- Excoffier L, Lischer HEL. 2010. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Mol Ecol Resour.* 10:564–567.
- Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Mol Mar Biol Biotechnol.* 3:294–299.
- Gable MF, Lazo-Wasem EA, Baldinger AJ. 2010. The Amphipoda of Bermuda – a century of taxonomy. *Zool Baetica.* 21:131–141.
- Gaillion B. 1828. Résumé méthodique des classifications des Thallassiophytes. *Dict Des Sci Nat.* 53:224–231.

- Gillespie RG, Roderick GK. 2002. Arthropods on islands: colonization, speciation, and conservation. *Annu Rev Entomol.* 47:595–632.
- Griffiths CL, Mead A, Robinson TB. 2009. A brief history of marine bio-invasions in South Africa. *Afr Zool.* 44:241–247.
- Healy B, Coates KA. 2003. A preliminary investigation of the decomposition of *Sargassum* stranded on Bermuda beaches, and the particular role of Enchytraeidae in its decomposition. *Newsl Enchytraeidae.* 8:13–24.
- Hearty PJ, Olson SL. 2010. Geochronology, biostratigraphy and changing shell morphology in the land snail subgenus *Poecilozonites* during the Quaternary of Bermuda. *Palaeogeogr Palaeoclimatol Palaeoecol.* 293:9–29.
- Hebert PDN, Penton EH, Burns JM, Janzen DH, Hallwachs W. 2004. Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly *Astraptes fulgerator*. *Proc Natl Acad Sci USA.* 101:14812–14817.
- Hedrick PW. 2005. Genetics of populations. 3rd ed. Boston (MA): Jones and Bartlett Publishers, Inc.
- Henzler CM, Ingolfsson A. 2008. The biogeography of the beachflea, *Orchestia gammarellus* (Crustacea, Amphipoda, Talitridae) in the North Atlantic with special reference to Iceland: a morphometric and genetic study. *Zool Scr.* 37:57–70.
- Ivanova NV, Dewaard JR, Herbert PDN. 2006. An inexpensive, automation-friendly protocol for recovering high quality DNA. *Mol Ecol Notes.* 6:998–1002.
- Knowlton N. 1993. Sibling Species in the Sea. *Annu Rev Ecol Syst.* 24:189–216.
- Knowlton N, Weigt LA. 1998. New dates and new rates for divergence across the isthmus of Panama. *Proc Roy Soc London, Series B.* 265: 2257–2263 [10.1098/rspb.1998.0568](https://doi.org/10.1098/rspb.1998.0568)
- Krøyer H. 1845. Karcinologiske Bidrag. *Naturhistorisk Tidsskrift.* 1:304. Fig.2.
- Kunkel BW. 1910. The Amphipoda of Bermuda. *Trans Connecticut Acad Arts Sci.* 16:1–116.
- LeCroy SE. 2011. An Illustrated Identification guide to the nearshore marine and estuarine gammaridean amphipoda of Florida. Vol. 5. Tallahassee, FL: Department of Environmental Protection; p. 739–763.
- Linnaeus C. 1758. *Systema Naturae Per Regna Tria Naturae, Secundum Classes, Ordines, Genera, Species, Cum Characteribus, Differentiis, Synonymis.* Copenhagen: Locis.
- Lobban CS. 1994. *Seaweed Ecology and Physiology.* Cambridge: Cambridge University Press; p. 366.
- Mateus A, Mateus E, Afonso O. 1986. Amphipodes littoraux et l'intérieur recueillies aux Açores pendant la campagne « Biazores » sur le navire Jean Charcot. *Anais Fac Ciñnce Porto.* 65:87–126.
- Morino H, Ortal R. 1993. The identity if *Talitroides alluaudi* (Chevreux) (Crustacea: Amphipoda: Talitridae) with notes on a new locality. *Proc Biol Soc Wash.* 106:332–338.
- O'Foighil D, Jennings R, Park J-K, Merriwether DA. 2001. Phylogenetic relationships of mid-oceanic ridge and continental lineages of *Lasaea* spp. (Mollusca: bivalvia) in the northeastern Atlantic. *Mar Ecol Prog Ser.* 213:165–175.
- O'Foighil D, Josefowicz CJ. 2009. Amphi-Atlantic phylogeography of direct-developing lineages of *Lasaea*, a genus of brooding bivalves. *Mar Biol.* 135:115–122.
- Olson SL, Hearty PJ. 2003. Probable extirpation of a breeding colony of short-tailed albatross (*Phoebastria albatrus*) on Bermuda by Pleistocene sea-level rise. *Proc Natl Acad Sci USA.* 100:12825–12829.
- Olson SL, Hearty PJ, Pregill GK. 2006. Geological constraints on evolution and survival in endemic reptiles on Bermuda. *J Herpetology.* 40:394–398.
- Pallas PS. 1766. *Miscellanea Zoologica quibus novae imprimus et obscurae animalium species describunter et observantionibus icomibusque illustrantur.* Berlin: Hagrae Comitum; p. P.119.
- Palmén E. 1949. *Talitroides alluaudi* (Chevreux) (Amphipoda, Talitridae) in Finland gefunden. *Archivum Societatis Zoologicae Botanicae Fennicae 'Vanamo'.* 2:61–64.
- Pavesi L, Wildish DJ, Gasson P, Lowe M, Ketmaier V. 2014. Further morphological and molecular studies of driftwood hoppers (Crustacea: Amphipoda: Talitridae) from Mediterranean/north-east Atlantic coastlines. *J Nat Hist.* doi:[10.1080/00222933.2014.974708](https://doi.org/10.1080/00222933.2014.974708)

- Radulovici AE. 2012. A tale of two biodiversity levels inferred from DNA barcoding of selected North Atlantic crustaceans. [Ph.D. thesis]. Rimouski (QC): University of Quebec at Rimouski; p. 245.
- Radulovici AE, Sainte-Marie B, Dufresne F. 2009. DNA barcoding of marine crustaceans from the Estuary and Gulf of St Lawrence: A regional-scale approach. *Mol Ecol Resour.* 9SUPPL. 1:181–187. doi:10.1111/j.1755-0998.2009.02643.x
- Ratnasingham S, Hebert PDN. 2007. BOLD: the Barcode of Life Data System: Barcoding. *Mol Ecol Notes.* 7:355–364.
- Ratnasingham S, Hebert PDN. 2013. A DNA-based registry for all animal species: the barcode index Number (BIN) system. *PLoS One.* 8:066213. doi:10.1371/journal.pone.0066213
- Rowe MP. 1998. An explanation of the geology of Bermuda Publ. Bermuda: Bermuda Government; p. 30.
- Say T. 1818. An account of the Crustacea of the United States (continued). *J Acad Nat Sci Philad.* 1:374–401.
- Shoemaker CR. 1935. The occurrence of the terrestrial amphipods, *Talitrus alluaudi* and *Talitrus sylvaticus*, in the United States. *J Washington Acad Sci.* 26:60–64.
- Stock JH, Biernbaum CK. 1994. Terrestrial amphipoda (Talitridae) from ascension and Saint Helena (South Central Atlantic). *J Nat Hist.* 28:795–811.
- Stoner AW, Greening HS. 1984. Geographic variation in the macrofaunal associates of pelagic *Sargassum* and some biogeographic implications. *Mar Ecol Prog Ser.* 20:185–192.
- Tamura K. 1992. Estimation of the number of nucleotide substitutions when there are strong transition-transversion and G+C-content biases. *Mol Biol Evol.* 9:678–687.
- Tamura K, Stecher G, Peterson D, Filipksi A, Kumar S. 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. *Mol Biol Evol.* 30:2725–2729.
- Thiel M, Haye PA. 2006. The ecology of rafting in the marine environment. III. Biogeographical and evolutionary consequences. *Oceanogr Mar Biol Ann Rev.* 44:323–429.
- Weir BS, Cockerham CC. 1984. Estimating F-statistics for the analysis of population structure. *Evolution.* 38:1358–1370.
- Wildish DJ. 2012. Long distance dispersal and evolution of talitrids (Crustacea: Amphipoda: Talitridae) in the northeast Atlantic islands. *J Nat Hist.* 46:2329–2348.
- Wildish DJ, LeCroy SE. 2014. *Mexorchestia*: a new genus of talitrid amphipod (Crustacea, Amphipoda, Talitridae) from the Gulf of Mexico and Caribbean Sea, with the description of a new species and two new subspecies. *Zootaxa.* 3856:555–577.
- Wildish DJ, Martell DJ. 2013. Digital recording of epidermal dorsal pigment patterns in amphipods (Crustacea). *Zoosyst Evol.* 89:311–316.
- Wildish DJ, Pavesi L, Ketmaier V. 2012. Talitrid amphipods (Crustacea: Amphipoda: Talitridae) and the driftwood ecological niche: a morphological and molecular study. *J Nat Hist.* 46:2677–2700.
- Zuill WS. 1999. The story of Bermuda and her people. London: Macmillan; p. 259.