Out of Alaska: morphological diversity within the genus *Eurytemora* from its ancestral Alaskan range (Crustacea, Copepoda)

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Abstract The copepod genus *Eurytemora* occupies a wide range of habitat types throughout the Northern Hemisphere, with among the broadest salinity ranges of any known copepod. The epicenter of diversity for this genus lies along coastal Alaska, where several species are endemic. Systematic analysis has been difficult, however, because of a tendency toward morphological stasis in this genus, despite large genetic divergences among populations and species. The goals of this study were to (1) analyze patterns of morphological variation and divergence within this genus, focusing on *Eurytemora* species that occur in North America, and (2) determine patterns of geographic and salinity distribution of *Eurytemora* species within the ancestral range in Alaska. We applied a comparative multivariate morphological analysis using 16–26 characters from 125 specimens from 20 newly collected sites in Alaska and 15 existing samples predominantly from North America. Results from principal component and hierarchical cluster analyses identified seven distinct morphological species of *Eurytemora* in North America (*E. affinis*, *E. americana*, *E. canadensis*, *E. composita*, *E. herdmani*, *E. pacifica*, and *E. raboti*), and identified diagnostic characters that distinguish the species (forming the basis for a new identification key). Several previously named species were regarded as synonyms. The sites we sampled in Alaska were remarkable in the high levels of sympatry of *Eurytemora* species, to a degree not seen outside of Alaska. Future studies of *Eurytemora* should shed light on patterns of habitat invasions and physiological evolution within the genus, and yield insights into mechanisms leading to its remarkably broad geographic and habitat range.

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Introduction

The copepod genus *Eurytemora* is prevalent throughout coastal regions of the Northern Hemisphere, across a remarkably broad range of habitats (Heron & Damkaer, 1976). Its latitudinal distribution spans from subtropical to subarctic, but with increasing species diversity toward northern latitudes. The region of Alaska and Northeastern Asia constitutes the epicenter of biodiversity for *Eurytemora* (Wilson & Yeatman, 1959; Johnson, 1961; Heron, 1964; Wilson & Tash, 1966; Heron & Damkaer, 1976), implicating this region as the evolutionary origin for this genus. Of the approximately 21 described species, most have been found in Alaska, and many are endemic to this region (Heron & Damkaer, 1976).

The genus *Eurytemora* is characterized by a broad habitat distribution ranging from the deep ocean, coastal marine, hypersaline salt marshes, and brackish estuaries, to completely fresh water (Heron & Damkaer, 1976; Lee, 1999, 2000; Gaviria & Forro, 2000). This genus might have the broadest salinity range of any copepod known (Heron & Damkaer, 1976). Several species of *Eurytemora* are particularly unusual in their broad salinity breadth, as most species of copepods are more restricted in their habitat distribution (Khlebovich & Abramova, 2000; Remane & Schlieper, 1971). Most notable is the species complex *Eurytemora affinis*, which has a geographic distribution that spans North America, Asia, and Europe, with habitat types that range from hypersaline salt marshes and brackish estuaries to completely fresh water (Saunders, 1993; Lee, 1999). Recent invasions by *E. affinis* from saline estuaries and salt marshes into inland freshwater lakes and reservoirs have been mediated by human activity (Saunders, 1993; Lee, 1999; Winkler et al., 2008). In general, 5–8 PSU defines a biogeographic and physiological boundary that separates fresh and saline invertebrates species (Hutchinson, 1957; Khlebovich & Abramova, 2000). Thus, members of the copepod genus *Eurytemora*, such as *E. affinis* and *E. velox*, are noteworthy in their ability to breach this major biogeographic boundary (Heron & Damkaer, 1976; Saunders, 1993; Lee, 1999; Gaviria & Forro, 2000).

However, patterns of habitat invasions and physiological evolution are difficult to probe in this genus, as taxonomic and systematic studies of *Eurytemora*

species are still in their incipient stages. Several species were described in the late nineteenth century (citations in Wilson & Yeatman, 1959), while a second wave of descriptions followed in the middle of the twentieth century (e.g., Heron, 1964; Wilson & Tash, 1966). Previous species designations within *Eurytemora* have been largely based on morphological descriptions of specimens from one or a few locations, such that there has been scant understanding of morphological variation within populations, or variation due to seasonal effects or geographic gradients. Although more than 20 nominal species have been proposed for the North American species, they have typically been studied in isolation, with little comparison among populations. Standard identification keys employ very few of the 20+ names; for instance, the Wilson & Yeatman (1959) key recognizes only four species, while the Pennak (1989) identifies only one species. Recent studies focusing on *E. affinis* have integrated analyses of molecular genetic divergence and reproductive isolation with traditional exoskeletal morphology (Lee, 2000; Lee & Frost, 2002). In this study, we applied comparative and comprehensive multivariate analyses of morphological traits for *Eurytemora* species to begin assessing systematic relationships among the species and to generate consistent and diagnostic identification keys.

A challenge in performing morphological systematics of this genus arises from morphological stasis (Lee & Frost, 2002). Morphological stasis appears to be a common problem in copepod systematics in general, where morphologically indistinguishable populations often show evidence of large genetic divergences and reproductive isolation (Carrillo et al., 1974; Lee & Frost, 2002; Dodson et al., 2003; Edmands & Harrison, 2003; Grishanin et al., 2005; Chen & Hare, 2008). For example, the copepod *Eurytemora affinis* exhibits rates of morphological evolution that are much slower than rates of molecular evolution (Lee & Frost, 2002). This pattern is evident from lower quantitative genetic (morphological) subdivision ($Q_{ST} = 0.162$) relative to molecular genetic subdivision ($G_{ST} = 0.617$) and lack of resolution in a morphological phylogeny of *E. affinis* populations relative to large molecular genetic divergences among clades (up to 20% sequence divergence in COI) (Lee & Frost, 2002). Reproductive isolation proved to be the most sensitive measure of species boundaries,

given the presence of reproductive isolation between morphologically indistinct and genetically proximate populations (Lee, 2000). Such cryptic species pose serious challenges for morphological systematics (Knowlton, 2000).

Another challenge in analyzing morphological variation arises from environmentally induced phenotypic plasticity. For example, *E. affinis* populations collected from the wild showed greater quantitative genetic (morphological) variance ($Q_{ST} = 0.292$, $N = 4$) than those reared in the laboratory ($Q_{ST} = 0.162$, $N = 4$) (Lee & Frost, 2002). In addition, Q_{ST} (morphological variance) increased with the inclusion of additional wild populations, while G_{ST} (molecular genetic variance) remained relatively constant. These results indicated that environmental factors could profoundly affect patterns of morphological variance due to phenotypic plasticity.

Despite the challenges mentioned above, studies of morphological traits provide a valuable initial step toward species identification and understanding of systematic relationships. As no previous study had performed an analysis of morphological traits of *Eurytemora* populations in a comprehensive and systematic manner, existing information is inconsistent and idiosyncratic (Dodson & Lee, 2006). Thus, the objective of this study was to elucidate and clarify patterns of morphological variation within North American species of the genus *Eurytemora*, focusing particularly within the ancestral range in Alaska. The specific goals of this study were to: (1) identify diagnostic morphological characters that could distinguish *Eurytemora* species, using principal component analysis, (2) determine hierarchical relationships among morphological species of *Eurytemora*, using hierarchical cluster analysis, and (3) uncover patterns of geographic distribution, habitat salinity, and co-occurrence of *Eurytemora* species within the ancestral range in Alaska. Examining populations within the ancestral range of the genus would potentially yield insights into the evolutionary history of this group. Morphological analyses were conducted concurrently with a molecular systematic study using 18S rRNA sequences (Skelly et al., in prep.). This study serves a foundation for future studies that explore patterns of habitat invasions and physiological evolution within the genus *Eurytemora*.

Materials and methods

Sampling locations

We sampled 54 locations in Alaska (Fig. 1) during June of 2005, primarily in the Northwest Arctic region near Kotzebue (66°53′N, 162°36′W). These sampling locations were chosen based on previous studies that found high diversity of *Eurytemora* species within this region (Wilson & Yeatman, 1959; Wilson & Tash, 1966; Heron, 1964). *Eurytemora* individuals were found in a wide range of salinities (0–28.8 PSU) and habitat types (Appendix Table 3 in Supplementary material). The Alaskan populations were collected using 53 μm plankton nets or 53 μm hand filters, and were preserved in ethanol within 8 h of collection. Water salinity was measured at each sampling site using a YSI conductivity meter. We also examined specimens from Alaska collected by Mildred Wilson and deposited in the collections of the National Museum of Natural History at the Smithsonian Institution, Washington, DC, USA.

In addition to the Alaskan samples, we examined additional *E. affinis* specimens, analyzed previously using molecular phylogenetic and morphometric analyses in Lee (2000) and Lee & Frost (2002). We included *E. affinis* specimens from the following populations: St. Lawrence River salt marsh, Quebec (Atlantic clade), St. John's River, Florida (Atlantic clade), Arbuckle Reservoir, OK (Gulf clade), Columbia River estuary, OR (North Pacific clade), Nitinat Estuary, BC, Canada (North Pacific clade) Nanaimo River, BC, Canada (North Pacific clade), and the northern Baltic Sea (Europe clade) (Appendix Table 3 in Supplementary material). In addition, we included *E. americana* specimens from the Duwamish River, WA (Lee, 2000) and Yaquina River, OR, USA. We also included specimens of *E. pacifica* collected from three locations in South Korea (Appendix Table 3 in Supplementary material), because *E. pacifica* had previously been found in Alaska (Heron, 1964), but was not present in our Alaskan samples. All specimens used in this study were from wild-caught samples rather than from specimens reared in the laboratory under constant conditions (see "Discussion"). Individual specimens and their measurements are listed in Appendix Tables 1 and 2 (Supplementary material).
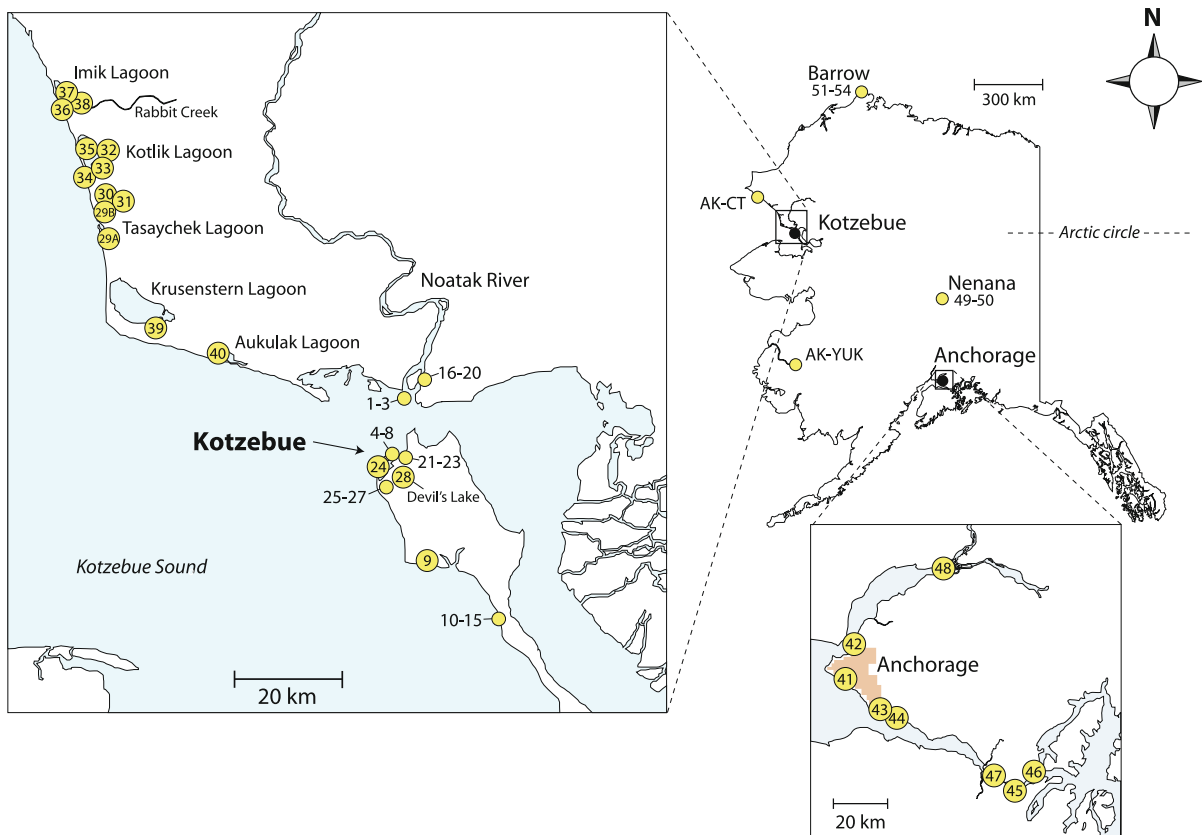
Fig. 1 Locations in Alaska sampled for this study. Locations in which *Eurytemora* species were found are shown in Fig. 6 and Appendix Tables 1 and 2 (Supplementary material). Details on sampling sites are described in Appendix Table 3

(Supplementary material). Numbers on the map correspond to those in Fig. 5 and Appendix tables (with AK prefix). AK-CT and AK-YUK are sampling locations of Mildred Wilson's collections at the Smithsonian Institution

Morphological characters

We used measurements of 26 characters from 125 female specimens to determine patterns of morphological variation and hierarchical clustering (see next sections). The morphological analyses were performed in two stages, where we first examined 16 characters used in previous studies of North American *Eurytemora* (e.g., used in Wilson & Yeatman, 1959), and then included an additional 10 characters to obtain greater resolution, for a total of 26 characters (Appendix Tables 1, 2 in Supplementary material). The analyses included only adult females (Fig. 2), because prior studies predominantly used female characters to identify *Eurytemora* species (Wilson & Yeatman, 1959; Pennak, 1989) and female structures (particularly the fifth legs) are flat and stiff, and therefore easier to measure accurately relative to the three-dimensional and softer male fifth legs.

For the first analysis, we measured 16 characters (see list below) for 74 individuals. We examined structures on one side of the body and on the first and fifth legs (P1 and P5), metasomal wing length, and length and width of the caudal ramus (Fig. 2; Appendix Table 1 in Supplementary material). Swimming legs and the calanoid fifth leg were coded as "P" and exopods were coded as "X" followed by the segment number. For example, P5X1 indicates the first (basal) exopod segment of the fifth leg.

All characters used in this study have been noted elsewhere, or at least illustrated (see citations in Table 2 for characters previously used to identify *Eurytemora* species). The lateral and terminal setae on the first swimming leg (P1) have been shown to be useful for taxonomic identification of many calanoid copepods (Park, 2000; Markhaseva & Ferrari, 2005). Changes in shape of the genital somite complex have been observed for many copepods (Park, 2000), and

Fig. 2 Morphological characters used in this study. The character codes (A1–A26) are described in the "Results" section
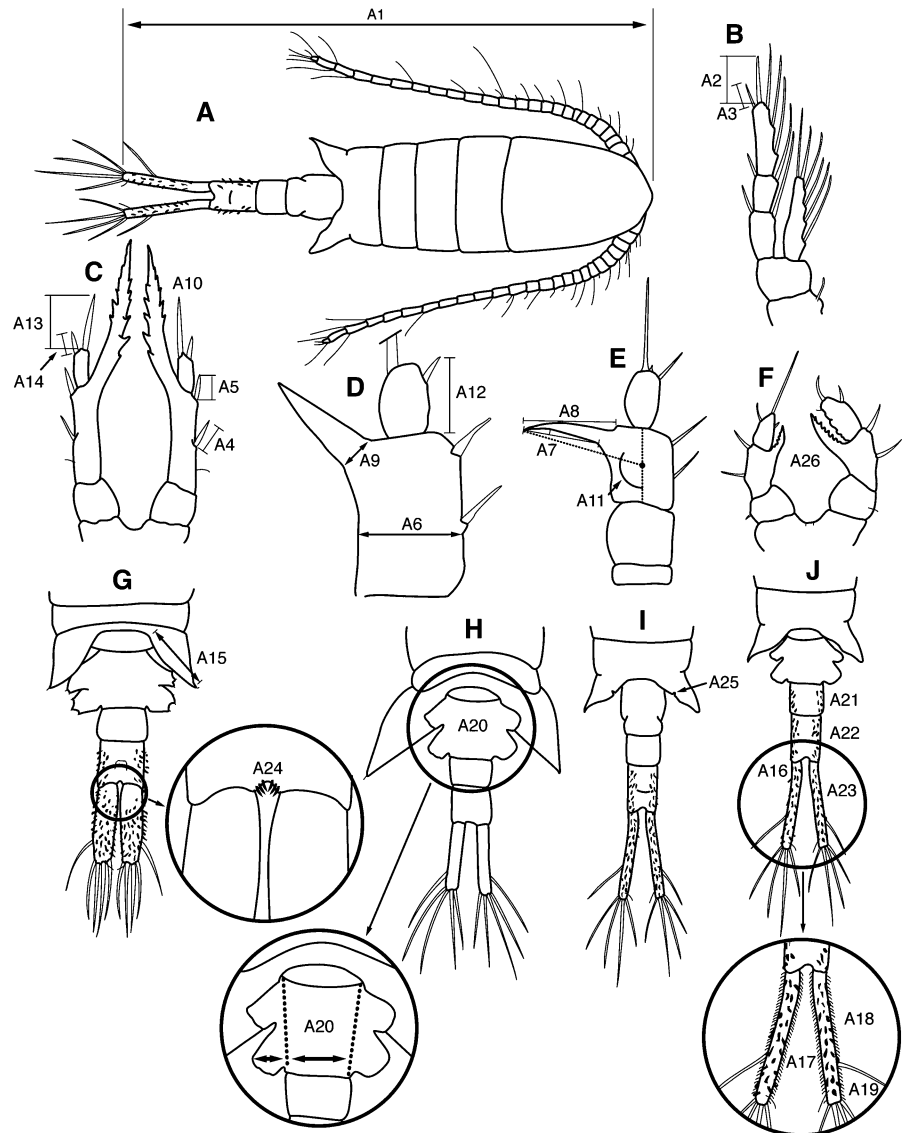
are assumed to reflect morphological divergence resulting from sexual selection during mating (Blades & Youngbluth, 1980). The internal projection (attenuation) on the proximal segmental complex of the exopod on the fifth leg (P5) is an apomorphy for the superfamily Centropagoidea to which *Eurytemora* belongs (Ferrari & Ueda, 2005).

Character Set 1 (16 characters)

A1 (Fig. 2A): Total body length, from the tip of the head to the end of the caudal ramus, not

including the caudal setae. Although body length is not in itself a useful character, it is often important to determine whether other characters vary with body length to detect allometric relationships.

A2 and A3 (Fig. 2B): Lengths of the terminal and subterminal setae of segment P1X3 (terminal segment).

A4 and A5 (Fig. 2C): Lengths of the proximal and distal lateral setae on segment P5X1.

A6 (Fig. 2D): Width of P5X1 measured at the level of the base of the proximal lateral seta.

A7 (Fig. 2E): Curvature of the internal projection of P5X1, measured as the distance between the

process and a line drawn between the projection tip and base. This character is used in Wilson & Yeatman (1959).

A8 (Fig. 2E): Length of the internal projection of segment P5X1 measured from the base (on the distal end of the segment) to the tip. There is often a joint, bulge, or wrinkle at the base of the projection, where it joins the segment.

A9 (Fig. 2D): Width of internal projection of segment P5X1, measured at the base of the projection. The basal thickness captures the impression that some projections are slender and some are wedge-shaped. This character is used in Wilson & Yeatman (1959).

A10 (Fig. 2C): The number of teeth on the internal projection of P5X1. Teeth can be present on one or both sides of the projection. In Fig. 2C there are 10 teeth on the projection, five on the distal (A10d) and five on the proximal (A10p) margin. In addition, the internal projection sometimes had a few hair-like microspinules (A10ms).

A11 (Fig. 2E): The angle of the internal projection on segment P5X1, relative to the long axis of the segment. Both the curvature and the angle measurements (A7 and A11) of the P5X1 internal projection were attempts to capture the distinction used by Wilson & Yeatman (1959): "inner process… …strongly directed backwards" versus "inner process…directed inwards".

A12 (Fig. 2D): Length of segment P5X2.

A13 and A14 (Fig. 2C): Lengths of the terminal and subterminal setae of segment P5X2. The relative lengths of the two apical spines is a character used in Wilson & Yeatman (1959).

A15 (Fig. 2G): Length of the metasomal wing, from the base at the internal medial angle to the tip.

A16 (Fig. 2J): The length of the caudal ramus measured along the outer margin.

For the second analysis, we used 51 additional specimens from Alaska and from additional North American sources, representing a wider range of *Eurytemora* forms (principally the M. S. Wilson collection, Smithsonian Institution and C. E. Lee's private collection), to analyze additional distinguishing characters (Appendix Table 2 in Supplementary

material). We could not use the 74 specimens described above as they were destroyed for DNA sequence analysis (Skelly et al., in prep.). We recorded data on 10 additional characters, such as ornamentation of the abdomen and caudal ramus, shape of genital segment and metasomal wings, and symmetry of P5 (A17–A26, Character Set 2 below). We also included four of the original 16 characters (A7, A9, A10, and A16 from Character Set 1 above) that were proven useful in the first set of analyses (see Table 1), and categorized them as discrete values as follows:

A7: 0 = negative, 1 = straight, 2 = positive

A9: 0 = slender, 1 = triangular, 2 = elongated

A10D: P5X1 internal process with teeth on distal margin = 1

A10ms: P5X1 internal process with teeth on proximal margin = 1

A10P: P5X1 internal process with microspines on proximal margin = 1

A16: The caudal ramus length was normalized by dividing it by the width of the ramus at the base, where it is widest, and this ratio was then categorized as 0 when <3.8, 1 when ≥3.8 and <4.0, and 2 when ≥4.0

Character Set 2 (10 characters)

A17 (Fig. 2J detail): Presence/absence of a row of microsetules along the internal margin of the caudal ramus. Do not confuse with spines from the dorsal patch.

A18 (Fig. 2J detail): A row of microsetules along the outer lateral margin of the caudal ramus, proximal from the lateral seta on the ramus: present/absent.

A19 (Fig. 2J detail): A row of microsetules along the outer lateral margin of the caudal ramus, distal to the lateral seta on the ramus: present/absent.

A20 (Fig. 2H detail): The ratio of the width of the genital segment divided by the length of the longest lateral lobe. The width of the segment is measured from lines drawn parallel to the body axis, from the corners of the segment. The length of the lobe is measured at right angles to the body axis. The ratio was

Table 1 Results of an iterative principal component analysis (PCA) of morphological characters from the first set of 74 specimens. N = the beginning number of specimens in each successive PCA, A = chance-corrected within-group agreement coefficient, and "P value" is the probability of A (McCune & Grace, 2002)

N	Number of outliers	*Eurytemora* outlier species	A	*P*-value	Distinguishing characters
74	8	*E. herdmani* (Heron, 1964)	0.239	≪0.001	A7, A8, A10, A11, and A15: relatively long P5X1 projection, which curves distally and has teeth on both sides (13–16 teeth total). Metasomal wings are relatively long
66	10	*E. canadensis* (Heron, 1964)	0.146	≪0.001	A10: 5–11 teeth on the distal margin of the P5X1 projection, which points medially from its segment. *E. herdmani* and *E. canadensis* are the only two forms with triangular teeth on the P5X1 projection
56	7	*E. raboti* (Heron, 1964)	0.209	≪0.001	The ordination separates out these 7 *E. raboti* specimens, but no single character is non-overlapping between the two groups. The characters, which together, separate out the seven specimens are A1, A4, A5, A8, A9, and A15: *E. raboti* were large specimens (1.52–2.36 mm compared to 1.16–1.92 mm for all other specimens) with long lateral setae on P5X1, long and wide P5X1 projection, and relatively long metasomal wings
49	9	*E. affinis* (Lee & Frost, 2002)	0.158	≪0.001	No single character separates out of these nine *E. affinis* specimens from the remaining 40 specimens. A combination is required of A6, A9, and A15: relatively wide P5X1 and P5X1 projection (compare Fig. 2D with Fig. 2E), long metasomal wings. Wilson (1959) described the P5X1 internal projection as "strongly directed backwards" [away from the body] in *E. affinis* and "directed inwards" in species in the *E. americana* group. In our data, the angle the projection points overlapped for the two species: between 120° and 135° in *E. affinis*, and between 96° and 135° in the *E. americana* group. Thus, the angle (A11) was not useful in separating the two groups. *E. raboti* and *E. affinis* are distinguished from the remaining specimens by the relatively wide projection of P5X1
40	12	*E. composita* (Wilson & Tash, 1966)	0.247	≪0.001	No single character separates these 12 *E. composita* specimens from the remaining 28 *E. americana* specimens. A combination is required of A5: long distal lateral seta on P5X1, the P5X1 projection often lacks hair-like microspinules
28		All 28 remaining in *americana* species group (see Johnson, 1961; Heron, 1964; Wilson & Tash, 1966)			

At each step of the iteration, specimens identified as an outlying group (Fig. 3) were removed, and the remaining specimens were again analyzed with PCA. The Multi-Response Permutation Procedure (MRPP) identified five distinct morphological species. Citations include recent descriptions

categorized as: 0 = 0.2, 1: >0.2 to <0.35, 2: ≥0.35 to <0.60, and 3: ≥0.60.

A21, A22, and A23 (Fig. 2J): Presence/absence of patches of microspines on the dorsal surface of the second and third abdominal segments and the caudal rami.
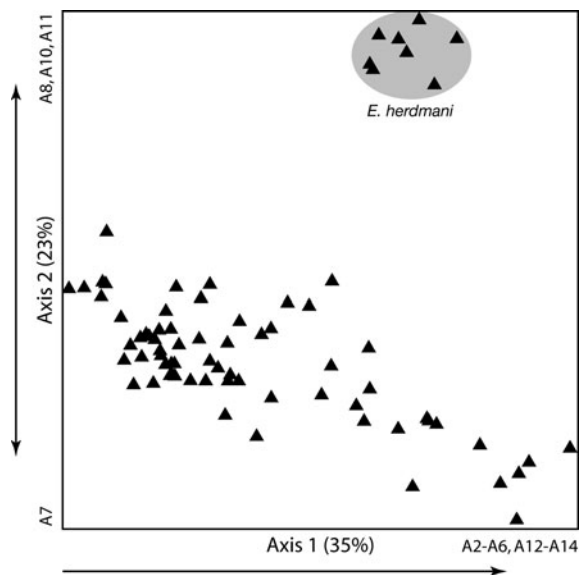
A24 (Fig. 2G detail): Presence/absence of stiff microspines on the posterior abdominal somite, between the two caudal rami.

A25 (Fig. 2I): Presence/absence of a notch near the base of the posterior margin of the metasomal wing.

A26 (Fig. 2F): Strong asymmetry of the left and right internal projections of segment P5X1 versus symmetrical projections. The projections were judged asymmetrical if one was at least 1.5 times as long as the other.

Ordination analyses

We performed multiple (serial) principal component analyses (PCA) using PC-ORD (McCune & Mefford, 2006). Ordinations were carried out on data after "general relativization" (McCune & Grace, 2002) that gave each variable equal means and equal weight for each character (the sum of values for each variable = 1.0 after relativization). Ordinations utilized Euclidean distances and a correlation cross-products matrix. Ordination analysis for the first set of 74 specimens revealed an outlier cluster of eight specimens (*E. herdmani* in Fig. 3). Significance of the outlier group was determined with a

Multi-response Permutation Procedure (McCune & Grace, 2002). The eight specimens were removed from the data set and identified to species (as *E. herdmani*). The remaining 66 specimens were analyzed again with PCA, revealing an outlier group of 10 individuals. This process was repeated until an ordination did not produce a significant outlier group. The iterative approach produced single clusters of outliers at each iteration of the analysis, as different characters were used at each iteration, separating out successive taxa.

Additional characters, not included in the first analysis (see previous section), provided greater resolution among species groups in a second specimen-by-morphology PCA analysis of 51 new specimens (Appendix Table 2 in Supplementary material). There were a total of 16 morphological variables (characters) in the second PCA (Appendix Table 2 in Supplementary material, where A1 was excluded). These characters were relativized, to remove undesired scale effects.

Hierarchical cluster analysis

We performed a hierarchical cluster analysis using the software package PC-ORD (McCune & Mefford, 2006) to obtain a dendrogram representing hierarchical relationships among morphological species of *Eurytemora*. The analysis was based on the first six principal components from the second ordination analysis of 51 specimens, where each principal component represented an independent character. That is, the input data for the hierarchical cluster analysis were scores of the first six PCA variates, based on a data matrix of 16 characters for 51 specimens (data shown in Appendix Table 2 in Supplementary material, with A1 excluded). Distance and linkage methods were chosen to minimize chaining (inappropriate sequential joining of individuals) (McCune & Grace, 2002). Using PC-ORD (McCune & Mefford, 2006), these requirements were met with a Euclidean distance measure and the flexible β ($\beta = -0.25$) group linkage method. Species can be misclassified because later fusions depend on earlier fusions (McCune & Grace, 2002). Therefore, the cluster analysis was followed up with a discriminant analysis (Minitab 15 Statistical Software, 2007) to identify potentially misclassified species.

Fig. 3 Principal component scatter plot from a PCA of 16 morphological characters measured for 74 *Eurytemora* specimens (Character Set 1, A1–A16; Appendix Table 1 in Supplementary material). The graph shows the first (axis 1) and second (axis 2) principal components. The cluster of points toward the top of the figure represents *E. herdmani*, based on the key in Heron (1964). Percentage variation accounted for by each principal component is shown in parentheses. Characters strongly correlated with the axes ($P < 0.01$) are listed along the relevant axis

Results

Ordination analysis

The first principal component analysis, using 16 morphological characters from 74 specimens (Appendix Table 1 in Supplementary material), identified six morphologically distinct groups that were identifiable as named species (Table 1; Fig. 3 shows the first cluster separating out). Strong correlations between characters and the principal components (axes) identified the most diagnostic characters for separating species (Fig. 3). The second principal component analysis (PCA) used diagnostic characters identified from the previous analysis and 10 additional characters (for a total of 16 morphological characters) from 51 specimens (Appendix Table 2 in Supplementary material). This second PCA identified additional diagnostic characters: A9, A10d, A10ms, A16, A18, and A22–A26 (Fig. 4). The first six axes (principle
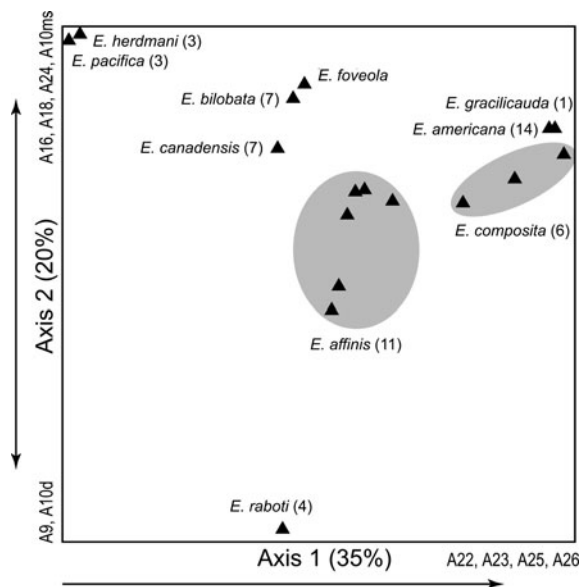
Fig. 4 Principal component scatter plot from a PCA of 16 morphological characters measured for 51 *Eurytemora* specimens (Character Set 2; Appendix Table 2 in Supplementary material; body size A1 was not used as a morphological variable in this analysis). The graph shows the first (axis 1) and second (axis 2) principal components. Numbers in parentheses indicate number of specimens used for each species. Percentage variation accounted for by each principal component is shown in parentheses along each axis. Characters strongly correlated with the axes ($P < 0.01$) are listed along the relevant axis

components) of the second PCA were used in the hierarchical cluster analysis to define morphological species (next section).

Hierarchical cluster analysis and identification of morphological species

A hierarchical cluster analysis using the first six axes (principal components) from a PCA of 16 characters (Appendix Table 2 in Supplementary material) for 51 specimens (McCune & Grace, 2002) identified seven major clusters, corresponding to seven known morphological species (*E. affinis*, *E. americana*, *E. canadensis*, *E. composita*, *E. herdmani*, *E. pacifica*, and *E. raboti*) (Fig. 5). The dendrogram (Fig. 5) showed a low level of chaining (12.1%). Discriminant analysis of the cluster data matrix (coded for seven species) did not identify misclassified items (species) (McCune & Grace, 2002).

Based on a literature review, we found that in several instances the same morphotype had been assigned multiple names, and several of the species names appeared to be synonymous with other forms (Table 2). For instance, a specimen that had been identified as *E. gracilicauda* (AK-MSW) appeared to be indistinguishable from *E. americana*, forming a cluster in the ordination analysis (Fig. 4) and hierarchical cluster analysis (Fig. 5). Two Alaskan samples labeled as *E. foveola* (AK-CT) and *E. yukonensis* by Mildred Wilson were nearly identical to each other. Based on the literature description, *E. yukonensis* appeared identical to *E. bilobata* (AK-YUK) (see Table 2). Specimens that had been identified as *E. foveola* (AK-CT) and *E. bilobata* (AK-YUK) did cluster with specimens of *E. affinis* in the hierarchical cluster analysis (Fig. 5), but were distant from other members of the *E. affinis* clade in the PCA (Fig. 4).

The cluster analysis was unable to resolve the sibling species structure within *E. affinis*. Previous studies have identified *E. affinis* as a sibling species complex, with genetically divergent clades and reproductive isolation among many of the populations (Lee, 2000; Lee & Frost, 2002). In our morphometric analysis, *E. affinis* populations formed two separate subclusters (Fig. 5), but this division did not correspond to any known genetic or geographic boundaries or patterns of reproductive isolation (Lee, 2000; Lee & Frost, 2002). For instance, both subclusters contained populations from the North
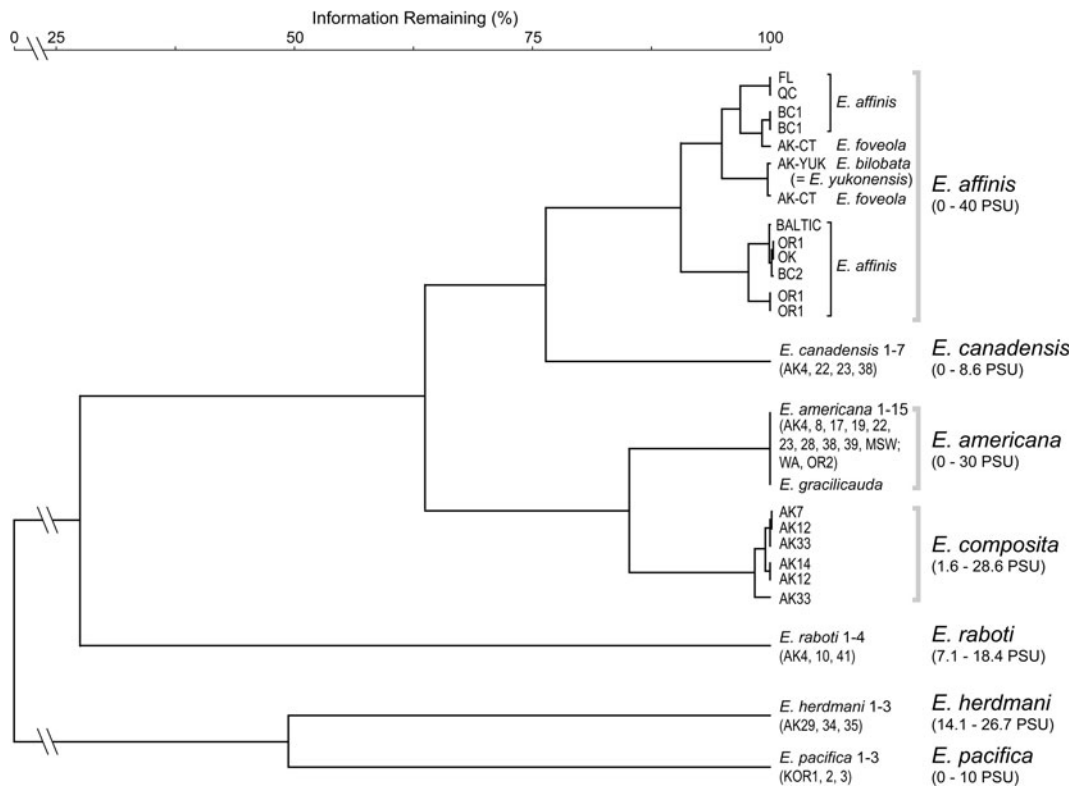
Fig. 5 Morphological dendrogram based on a hierarchical agglomerative cluster analysis (McCune & Grace, 2002) using scores of the first six principal components from a PCA of 16 morphological characters from 51 specimens (Character Set 2; see "Materials and methods" section). Specimens used for this analysis are shown at the branch tips, indicated by sampling locations of the specific specimens (Fig. 6 for Alaska samples (prefixed by "AK"); Appendix Table 3 in Supplementary material). The seven species names recognized in this study are on the far right. The scale above the graph indicates the amount of information (related to the total morphological variance) consumed by the progressive agglomerations. Salinity ranges of samples (across locations) are indicated under the species name

Pacific clade, which was identified by COI nucleotide sequences (Lee, 2000).

Geographic and salinity distribution

Of the 54 locations that we sampled in Alaska (Fig. 1), we found members of the genus *Eurytemora* in 19 sites along the coastal zones of Alaska (Fig. 6). *Eurytemora* specimens were not found in 35 sites, including locations near Anchorage [Fig. 1, sites 42–48; Portage Glacier, Prince William Sound (near Whittier), Potter's Marsh, Westchester Lagoon, and Knik River] and near Nenana (Fig. 1, sites 49, 50; Tanana River). The greatest number of species were found in the vicinity of Kotzebue (Fig. 6), a region previously reported to be rich in *Eurytemora* species (Heron, 1964). While there was much overlap in distribution among *Eurytemora* species, especially in the

Kotzebue region (Fig. 6), we did find evidence of geographic structure. In general, we found a northward distribution of *Eurytemora herdmani*, a southward distribution of *Eurytemora raboti*, and overlapping distributions of *E. americana*, *E. canadensis*, and *E. composita* centered around Kotzebue (Fig. 6).

The area directly east of the city of Kotzebue proper (Fig. 6, sites 4, 7, 8) contained the highest diversity of *Eurytemora* species in this study (five species), in a series of shallow small ponds (near Ted Stevens Way Bridge). Brackish lagoons east of Kotzebue proper (sites 22, 23) contained three *Eurytemora* species. Devil's Lake to the southeast, a large freshwater drinking water reservoir, contained *E. americana* (site 28). Directly south of Kotzebue was an extensive low-elevation mud flat (sites 12, 14; Riley's Wreck) with many shallow pools of relatively high salinity, many of which were ephemeral, and

Table 2 Descriptions of North American species of *Eurytemora*

Species	Original citation	Annotation
E. affinis	Poppe 1880	A species complex characterized by morphological stasis, but with large molecular divergence among geographically distributed clades (Lee, 2000; Lee & Frost, 2002). Populations (sibling species) occur throughout the Northern Hemisphere. *E. affinis* is widespread in North America and northern Europe, and also occurs in Asia. The morphological dendrogram shows *E. bilobata* Akatova 1949 and *E. foveola* clustering within the *E. affinis* clade (Fig. 5). A specimen of "*E. yukonensis*" Wilson 1951 from the US Smithsonian M. S. Wilson collection (sample Y522) appears identical to the *E. bilobata* specimen
		The European (Scandinavia, Russia) *E. grimmi* Sars 1897 has an *E. affinis*-like P5; but its P5X1 has only one lateral seta; all other *E. affinis* forms except some *E. velox* have two lateral setae on P5X1. *E. grimmi* lacks feathered setae on the inner margin of coxopodite on P1–P5 (all others have feathered setae), and P5N1 has five setae (all others have six setae). *E. hirundo* and *E. hirundoides* (from Europe and northern Asia) are synonymous with *E. affinis* (Heron & Damkaer, 1976; Lee, 2000)
E. americana	Williams 1906	Synonyms include *E. thompsoni* Willey 1923, *E. transversalis* Campbell 1930 and *E. kieferi* Smirnov 1931 (Gardner & Szabo, 1982). *E. arctica* Wilson & Tash 1966 is described as being similar to *E. composita* Keiser 1929 and *E. gracilicauda* Akatova 1949, except that *E. arctica* has an "unusually long" internal projection on P5X1 (Wilson & Tash, 1966). The morphological dendrogram (Fig. 5) shows *E. gracilicauda* clustering with the *E. americana* specimens. This specimen, identified by M. S. Wilson (Smithsonian Collection) appears to be an unusually large (1.72 mm) *E. americana*
		We suspect that *E. arctica* is also a synonym of *E. americana*. The name *E. americana* is not used in Wilson & Tash (1966), and we suspect that *E. arctica* they describe is equivalent to *E. americana*. There were only immature females in the US Smithsonian Institution sample "MS Wilson A1030(1) Lake #4, Cape Thompson area Aug. 2, 1961 Tash #409 *Eurytemora arctica* 28CV female." *E. arctica* has been found in the Cape Tompson, Umiat, and Noatak areas of NW Alaska (Wilson & Tash, 1966). The distribution of *E. arctica* lies within the range of *E. americana* reported by Gardner & Szabo (1982)
		The European and Russian *E. gracilis* Sars 1898 is similar to the North American *E. americana* (based on the illustration in Borutzky et al., 1991). The form is characterized only as having triangular metasomal wings, which is not very descriptive. *E. anadryensis* Borutsky 1961 is considered as a synonym of *E. gracilis* (Walter, 2010)
E. canadensis	Marsh 1920	Wilson & Yeatman (1959) consider *E. tolli* Rylov 1922 as the same form
E. composita	Keiser 1929	Similar to *E. americana*, except the genital segment lobes are larger, and the dorsal spine patches on the second abdominal segment are unique in the genus (Wilson & Yeatman, 1959)
E. herdmani	Thompson & Scott 1897	Heron (1964) also found this form along the coast of NW Alaska
E. pacifica	Sato 1913	Johnson (1961), Heron (1964), and Gardner & Szabo (1982) consider *E. johanseni* Willey 1920 to be a synonym of *E. pacifica*. We did not observe *E. pacifica* in our samples from Alaska, but it is reported from Alaska (Johnson, 1961; Gardner & Szabo, 1982) and the northern Pacific coast of Asia (e.g., Korea). Our analysis is based on specimens from South Korea. Illustrations of *E. asymmetrica* Smirnov 1935 suggest that it might be the same as *E. pacifica*
E. raboti	Richard 1897, Behm 1917	Heron (1964) also collected this species. It has a P5X1 similar to that of *E. affinis*, but the ornamentation of the abdomen and caudal rami are distinctive, as are the unusually short caudal rami

full of *Eurytemora* (*raboti* and *composita*). Along the Noatak River, large permanent low-salinity ponds (sites 17, 19) contained *E. americana*. North of Kotzebue, within Cape Krusenstern National Monument, more saline coastal lagoons separated from ocean by only a narrow barrier (sites 29, 34, 35) contained only *E. herdmani*. Lower salinity ponds adjacent to the lagoons (sites 33, 39, 38) contained additional species (*E. americana*, *E. canadensis*, and *E. composita*).
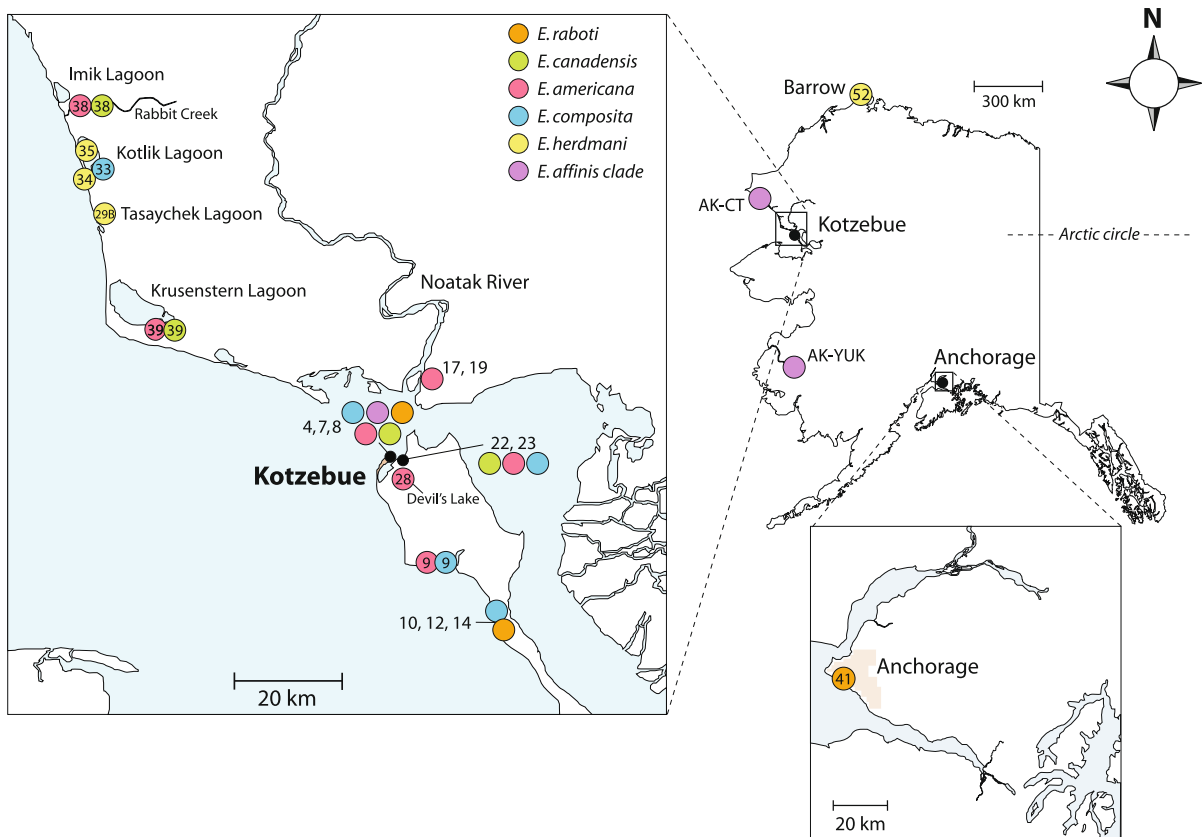
Fig. 6 Locations of *Eurytemora* species found in this study. *Colors* correspond to six of the seven morphological species of *Eurytemora* defined in this study. Numbers refer to sites where *Eurytemora* species were found (also indicated Appendix Tables 1, 2 in Supplementary material) among the sites that were sampled (Fig. 1). Salinity and specific locations of sites are described in Appendix Table 3 (Supplementary material). "*E. affinis* clade" (*purple*) refers to specimens of *E. foveola* and *E. bilobata*, which clustered with the *E. affinis* clade (Fig. 5). AK-CT and AK-YUK refer to Mildred Wilson's collections at the Smithsonian Institution (Appendix Table 2 in Supplementary material)

Salinity was not significantly correlated with any of the axes in the second PCA of 51 specimens, indicating no correlation with morphology. Within Alaska, *E. americana*, *E. canadensis*, and *E. affinis* clade (*E. foveola* and *E. bilobata*) were found in the lower salinity ranges (fresh-brackish), while *E. composita*, *E. raboti*, and *E. herdmani* were found in higher salinity ranges (brackish-marine). Within Alaska, *E. americana* was found in habitats that were considerably more fresh (0–8.6 PSU; Fig. 5, Appendix Table 3 in Supplementary material) than its salinity distributions reported outside of Alaska, where it is found in more saline portions of the estuary (25–30 PSU) relative to *E. affinis* (see Heron & Damkaer, 1976; Lee, 1999). In contrast, *E. herdmani* occupied the more saline lagoons in Alaska (14–27 PSU) at a salinity range similar to that found outside of Alaska (15–35 PSU) (George, 1985; Winkler et al., 2008).

Co-occurrence of *Eurytemora* species and body size displacement

There was a considerable degree of overlap in range among the *Eurytemora* species (Fig. 6; Appendix Tables 1, 2 in Supplementary material). Among species found in Alaska, only *E. herdmani* did not co-occur with any other *Eurytemora* species. In contrast, other *Eurytemora* species tended to show a large degree of cohabitation in Alaska (Fig. 6). For

example, *E. americana* co-occurred in six locations with *E. canadensis* (sites 4, 7, 22, 23, 38, 39), four with *E. composita* (sites 4, 7, 9, 23), one with *E. raboti* (site 4), one with *E. foveola* (*E. affinis* clade, site 8), and was found alone only at three sites (sites 17, 19, 28). The sites directly east of Kotzebue proper (sites 4, 7, 8) tended to have the highest species diversity, with four species occurring in site 4 (*E. americana*, *E. canadensis*, *E. composita*, and *E. raboti*), three species in site 7 (*E. americana*, *E. canadensis*, and *E. composita*), and two species in site 8 [*E. americana* and *E. foveola* (*E. affinis* clade)].

The species of *Eurytemora* exhibited significant differences in body size in samples in Alaska (ANOVA; $F = 16.61$, df = 4, $P < 0.0001$), showing size partitioning among *E. raboti* (2.02 mm ± 0.20 SE), *E. canadensis* (1.77 mm ± 0.042 SE), *E. americana* (1.57 mm ± 0.030 SE), *E. composita* (1.41 mm ± 0.040 SE), and *E. herdmani* (1.29 mm ± 0.070 SE). *E. americana* and *E. canadensis* differed significantly in a body size in sympatry (Student's $t = -4.27$, df = 10, $P = 0.0016$), with a ratio of 1.14 (where *E. canadensis* was larger). Moreover, *E. americana* exhibited greater body length when it occurred alone than in sympatry with *E. canadensis* (one-tailed t test; $t = -2.35$, df = 7, $P = 0.026$). Body size also differed significantly between *E. americana* and *E. composita* where they were found together (Student's $t = 3.13$, df = 6, $P = 0.020$), with a body size ratio of 1.11 (where *E. americana* was larger). When alone, *E. composita* was larger than when found with *E. americana*, but the differences were not significant (one-tailed t test; $t = 2.10$, df = 5, $P = 0.15$).

Discussion

The epicenter of diversity for the genus *Eurytemora* lies within Alaska, where the majority of the 21 previously recognized species have been known to occur (Heron & Damkaer, 1976). In his 1958 address to the Society of American Naturalists, *Homage to Santa Rosalia or why are there so many different kinds of animals?*, G. Evelyn Hutchinson noted the importance of niche diversity and allopatric speciation (followed by secondary contact) for generating species diversity (Hutchinson, 1959). Habitats of the genus *Eurytemora* in its ancestral range are

characterized by instability, with a filigree of diverse habitat types dotting a coastal landscape. Fluctuating conditions over glacial cycles might have promoted the evolution of multiple species in allopatry followed by secondary contact on a repeated basis, creating multiple species within a limited geographic region.

Patterns of morphological divergence within the genus *Eurytemora*

Inconsistencies among previous studies have rendered species designations uncertain for the genus *Eurytemora*. Our analysis identified seven morphological species within the genus (*E. affinis*, *E. americana*, *E. canadensis*, *E. composita*, *E. herdmani*, *E. pacifica*, and *E. raboti*). A comprehensive multivariate approach, applying ordination (PCA) and hierarchical cluster analyses, identified the seven distinct species based on 26 morphological characters from 125 female specimens (34 locations) (Figs. 4, 5). As a result, our morphological dendrogram (Fig. 5) represents the first hierarchical reconstruction of species relationships for the genus *Eurytemora*, and provides a foundation for future studies that explore evolutionary relationships, physiological ecology, and patterns of speciation for the genus. As this study focused on North American species of *Eurytemora*, our analysis did not include species that occur exclusively in Europe, such as *E. lacustris*, *E. velox*, and *E. grimmi*, or the deep water Asian *E. richingsi* (Heron, 1964; Heron & Damkaer, 1976). With the inclusion of these other species, our analysis would represent a major step toward revising the genus (identification keys are provided in the "Identification key for adult females of North American *Eurytemora*" section).

In addition to the seven species identified by our analyses, at least 14 additional names had been assigned to North American specimens, many of which we now consider synonyms of other forms (Table 2). In our morphological analysis, a specimen that had been labeled as *E. gracilicauda* was indistinguishable from *E. americana* (Fig. 5). Likewise, specimens that had been attributed to *E. bilobata* Akatova 1949 (=*E. yukonensis* Wilson 1951) and *E. foveola* Johnson 1961 were morphologically very close to one another (Fig. 4). While *E. bilobata* and *E. foveola* were outliers from other members of the *E. affinis* clade in the PCA (Fig. 4), they formed a

clade with *E. affinis* in the hierarchical cluster analysis based on 16 morphological traits (Fig. 5). In addition, *E. foveola* did form a clade with *E. affinis* in a molecular phylogeny based on 18S rRNA sequences, but was genetically more divergent (showing long branch length) relative to other members of the clade (Skelly et al., in prep.). Specimens that had been identified as *E. foveola*, *E. bilobata*, and *E. yukonensis* (by M. S. Wilson) might represent genetically distinct clades, or paraphyletic species, within the *E. affinis* species complex. However, further investigation into genetic relationships and reproductively isolation would be required to determine how these forms are related to *E. affinis*.

Our morphometric analysis was unable to resolve hierarchical relationships among genetically divergent clades within the *E. affinis* species complex (Lee, 2000). Patterns of morphological divergence within *E. affinis* did not reveal any meaningful structure (with individuals from the same population appearing in different clusters) (Fig. 5). Morphological stasis in relevant diagnostic characters along with morphological plasticity of environmentally influenced traits might have both contributed to this lack of resolution (Lee & Frost, 2002). Environmental plasticity is likely to have affected our morphometric analysis, given that our measurements were based on specimens collected from the wild, rather than on laboratory-reared animals. This problem might have been more acute for *E. affinis* than for the other *Eurytemora* species, as *E. affinis* specimens were included from a much broader geographic and habitat range than for other species (Appendix Table 3 in Supplementary material). The lack of resolution found here for *E. affinis* clades was concordant with a previous morphological study that used eight secondary sex characters, except that the previous study was able to differentiate the European clade from the other *E. affinis* clades (Lee & Frost, 2002). This previous study included male secondary sex characters (male fifth leg and antennules), indicating that the inclusion male characters would not provide adequate resolution to distinguish among *E. affinis* clades.

Morphological stasis and morphological plasticity do pose limitations on the morphological classification of copepods. In general, morphological divergences provide much less resolution for distinguishing sibling species within *Eurytemora* than molecular phylogenies or patterns of reproductive isolation (Lee, 2000;

Lee & Frost, 2002; Skelly et al., in prep.). Many copepod species tend to exhibit morphological stasis, where populations are indistinguishable based on morphological characters, while showing large genetic divergences and reproductive isolation among populations (Carrillo et al., 1974; Dodson et al., 2003; Edmands & Harrison, 2003; Grishanin et al., 2005; Chen & Hare, 2008).

However, despite these limitations, morphological data do provide a rich resource for examining patterns of evolution and speciation, in conjunction with data on molecular divergences and reproductive isolation (Lee & Frost, 2002). The morphological species we define here are likely to reflect evolutionary relationships among real categories, and will be contrasted with a molecular phylogeny of this group in a companion study (Skelly et al., in prep.).

Geographic distribution of the genus *Eurytemora* in Alaska

G. Evelyn Hutchinson once remarked that "…the process of natural selection, coupled with isolation and later mutual invasion of ranges leads to the evolution of sympatric species, which at equilibrium occupy distinct niches…" (Hutchinson, 1959). The region in Alaska that we sampled for this study was remarkable in the high degree of sympatry of *Eurytemora* species (Fig. 6). The Kotzebue region in Alaska includes six of the seven Alaskan *Eurytemora* species that we recognize (Fig. 5), comprising about half of the species in the genus *Eurytemora* if we include the synonyms (Table 2). High species diversity of *Eurytemora* had been discovered in other regions of Alaska, such as Cape Thompson, where six of the species defined by this study (Fig. 5) could be found (Wilson & Tash, 1966). Of the species recognized in our morphological analysis (Fig. 5), four are found outside of Alaska (*E. affinis*, *E. americana*, *E. herdmani*, and *E. pacifica*). These four species are not each other's closest relatives based on the morphological dendrogram (Fig. 5) and a molecular phylogeny based on 18S rRNA sequences (Skelly et al., in prep.), indicating multiple independent dispersal events out of Alaska. *Eurytemora* species have a tendency toward cold adaptation, such that species ranges beyond Alaska might be limited by temperature (Katona, 1970).

This coastal region of Alaska that comprises the ancestral range for this genus is striking in its complexity, diversity, and instability of habitats. The high density of microhabitats in this region might have allowed a diversity of *Eurytemora* species to evolve in semi-isolation, followed by subsequent merging of habitats, and secondary contact of species. Most remarkable was the area directly east of the city of Kotzebue proper (Fig. 6, sites 4, 7, 8), where a complex filigree of numerous low-lying ponds contained the highest diversity of *Eurytemora* species in this study (five species). These ponds, on peat soil, varied in salinity and appeared to be in a dynamic state of flooding and merging. Many of the habitats with high diversity of *Eurytemora* species were ephemeral and characterized by unstable and fluctuating conditions.

Body size displacement in sympatry

Four species of *Eurytemora* showed a consistent gradient in body size where they co-occurred (in descending order: *E. raboti*, *E. canadensis*, *E. americana*, and *E. composita*). Hutchinson & MacArthur (1959) proposed that congeneric species in sympatry would exhibit differences in body size, reflecting "ecological character displacement," to reduce competition for limited resources (Brown & Wilson, 1956; Hutchinson & MacArthur, 1959; Schluter, 2000). For co-occurring species of mammals and birds Hutchinson found that the ratio of sizes varied from 1.1 to 1.4, with the mean ratio being 1.28, and a minimum difference in the ratio of body sizes of at least the cube root of 2, or 1.26 between predators and prey (Hutchinson, 1959). Body size ratios of 1.14 between *E. canadensis* and *E. americana* and of 1.11 between *E. americana* and *E. composita* were within the range of Hutchinson's empirical observations of 1.1–1.4 across a wide range of taxa (Hutchinson, 1959). In addition, *E. americana* appeared to be smaller in the presence of *E. canadensis* than when found alone (see "Results" section).

However, observations of body size from field-caught samples cannot reveal whether body size displacement is the result of phenotypic plasticity or selection. Body size displacement according to Hutchinson's hypothesis would be a consequence of natural selection acting to reduce competition between species. There is evidence for heritability of body size in *E. herdmani*, but the trait is also subject to plasticity (McLaren, 1976). A common-garden experiment would be required to determine the degree to which body size differences among *Eurytemora* species in sympatry are due to heritable differences, rather than plasticity. In addition, it is not clear whether body size displacement would really be linked to competition between species. If competition for food were the important factor, differences in particle capture size by the maxillae of the *Eurytemora* species might be a more relevant measure than body size. Moreover, what body size ratios actually indicate and whether they reflect phenomena of biological consequence have been subjects of debate (Dodson, 1974; Horn & May, 1977; Simberloff & Boecklen, 1981; Eadie et al., 1987).

Salinity distribution of *Eurytemora* species

Despite the physiological barrier that tends to separate freshwater and saline invertebrate species (Hutchinson, 1957; Khlebovich & Abramova, 2000), the genus *Eurytemora* has among the broadest salinity distributions known for a copepod (0–40 PSU) (Heron & Damkaer, 1976; Lee, 1999). Our sampling revealed a broad salinity distribution for many species of *Eurytemora*, as well as variation in salinity distribution among species (Fig. 5; Appendix Table 3 in Supplementary material).

For *E. americana*, salinity distributions we found within Alaska differed sharply from that found outside of Alaska. Within Alaska, *E. americana* was found at very low salinities (0–8.6 PSU), including in fresh water (Fig. 6; Appendix Table 3 in Supplementary material) (Wilson & Tash, 1966). Yet, outside of Alaska this species has not been found in fresh water and tends to have a more saline distribution than *E. affinis*, in the 20–30 PSU portion of estuaries (Jeffries, 1962; Heron & Damkaer, 1976). The lower salinity range of *E. americana* within Alaska might partly be a consequence of displacement by the other co-occurring *Eurytemora* species (*E. composita* and *E. raboti*). Absence of these species outside of Alaska might allow *E. americana* to occupy more saline distributions.

Eurytemora is likely to constitute a highly evolvable genus with respect to salinity (for a discussion

on evolvability see Lee & Gelembiuk, 2008). Glacial cycles and coastal disturbances might have resulted in multiple incursions into differing salinities over evolutionary time. Though, it is unclear whether the ancestral state for the genus is saline or fresh. *Eurytemora* belongs to the family Temoridae, which includes genera that are both marine and freshwater. However, *Eurytemora* is considered most closely related to the genus *Temora*, which is strictly marine (Gurney, 1931).

Transitions between saline and freshwater habitats for species of this genus likely entail evolutionary responses, as physiological experiments have shown that the transition to fresh water by *E. affinis* requires a response to selection (Lee et al., 2003, 2007). Survival at fresh water is negatively genetically correlated with survival at 5 PSU and higher salinities, which indicates that selection for freshwater tolerance would select against high salinity tolerance (Lee et al., 2003, 2007). It is intriguing that different populations and species of *Eurytemora* do vary in their salinity breadth (Heron & Damkaer, 1976; Lee, 1999; Lee et al., 2003; Winkler et al., 2008; Skelly et al., in revision), and that some populations of *E. affinis* appear to have a much greater ability to invade habitats of different salinity than others (Lee, 1999; Skelly et al., in revision). It would be intriguing to explore the evolutionary mechanisms that underlie differences in geographic and habitat distributions among "species" of *Eurytemora*, and the ability of some populations to readily expand their ranges into novel habitats (Lee & Gelembiuk, 2008).

Identification key for adult females of North American *Eurytemora*

The following identification key incorporates several new characters. These characters, through their strong correlation with PCA axes, were identified as the best choices for distinguishing the species.

1A: P5X1 internal projection with at least eight teeth along one or both margins (Fig. 2C, F); teeth are triangular, with smooth or serrated margins, and three to five times as long as wide…………2
1B: P5X1 internal projection with fewer than eight teeth (Fig. 2D, E) although hair-like microsetules may be present on one or both sides of the projection margin)………………………………4

2A (from 1A): P5X1 internal projection with a total of 10–16 teeth, teeth on both sides of the projection, projection curved to be nearly parallel to segment (Fig. 2C character A10)………………*E. herdmani* Thompson & Scott 1897
2B: P5X1 internal projection with a total of 8–11 teeth, teeth on only one (distal, external) margin of the projection (Fig. 2F)…………………………..3
3A (from 2B): P5 right and left distal segments and terminal setae symmetrical, projections nearly perpendicular to the long axis of the segment……………*E. canadensis* Marsh 1920
3B: P5 right and left distal segments and terminal setae asymmetrical, projections from P5X1 nearly parallel to the long axis of the segment (Fig. 2F)………………*E. pacifica* Sato 1913
4A (from 1B): Genital segment with two lobes on each side, maximum lobe length >60% as long as the width of the segment; caudal rami 3.5–3.6 times as long as wide (Fig. 2G)………………*E. raboti* Richard 1897
4B: Genital segment lobes <50% as long as the width of the segment; caudal rami 4.8–6.6 times as long as wide (as in Fig. 2I)………………………5
5A (from 4B): Patches of microspinules present on the dorsal surface of abdominal segment 2 (Fig. 2J, character A21)………………*E. composita* Keiser 1929
5B: Abdominal segment 2 lacking patches of microspinules…………………………………….6
6A (from 5B): P5X1 projection wedge-shaped or triangular (Fig. 2D), with straight sides, and the tip of the projection is as far distal as the tip of P5X2………………*E. affinis* Poppe 1880 (This species group includes Alaskan populations identified by M. S. Wilson as *E. bilobata* and *E. foveola*. Both *foveola* and *bilobata* lack dorsal microspine patches on abdominal segments 2 and 3, and on the caudal rami. The *affinis* forms have microspine patches on the rami and at abdominal segment 3)
6B: P5X1 projection slender (Fig. 2E) with a concave proximal margin, sides curved (species in the *americana* and *bilobata* groups), and the tip of the projection is only as far distal as the base of P5X2………………*E. americana* Williams 1906………………(This species group includes Alaskan specimens identified by M. S. Wilson as *E. gracilicauda*)

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