Biological Journal of the Linnean Society, 2006, **89**, 469–488. With 3 figures

The systematics of Australian *Daphnia* and *Daphniopsis* (Crustacea: Cladocera): a shared phylogenetic history transformed by habitat-specific rates of evolution

J. K. COLBOURNE[1]*, C. C. WILSON[2] and P. D. N. HEBERT[3]

[1]*Center for Genomics and Bioinformatics, Indiana University, Bloomington, IN 47405, USA*
[2]*Aquatic Biodiversity and Conservation, OMNR, Trent University, Peterborough, Ontario K9J 8 N8, Canada*
[3]*Department of Integrative Biology, University of Guelph, Ontario N1G 2W1, Canada*

Received 2 March 2005; accepted for publication 15 January 2006

This study examines the molecular-genetic divergence and evolution of Australian aquatic micro-Crustacea *Daphnia* and *Daphniopsis*. The results indicate that species of *Daphniopsis* are accommodated within the genus *Daphnia*. Although their phyletic integrity is no longer supported, all *Daphniopsis* species (save one from North America) form a monophyletic group and may warrant subgeneric recognition pending further systematic investigations. A total of five lineages are shown to occupy Australian inland waters, including an endemic subgenus (*Australodaphnia*) and representatives of the subgenus *Ctenodaphnia*. The subgenera (*Daphnia* and *Hyalodaphnia*) that dominate the North American fauna are absent in Australia. The large extent of sequence divergence among major groups suggests that continental isolation has helped shape the early evolution of daphniids. More recent speciation is also evident, particularly by the *Daphnia carinata* species complex, whose numbers have grown to 13 members by the addition of a species previously assigned to the nominal subgenus and species yet to be formally described. The molecular data provide more evidence that the colonization of distinct habitats and ecological settings is a key factor in spurring diversification in the genus, while also modulating the pace of molecular evolution. This study attributes habitat-specific molecular clocks to the intense ultraviolet (UV) exposure in both saline and transparent oligohaline waters. Adaptations to these harsh environments by at least four independent lineages include the convergent acquisition of a melanic carapace. Yet some lineages, clearly under mutational duress, lack this commonly acquired protective trait. There are numerous adaptive lines of defense against UV damage, including the complex regulatory mechanisms required to initiate a cellular response to guard and repair DNA. Functional molecular studies may soon challenge a notion built on morphology that convergence is the general directive to *Daphnia*'s ecological and evolutionary success. *Biological Journal of the Linnean Society*, 2006, **89**, 469–488.

ADDITIONAL KEYWORDS: ecology – molecular evolution – mtDNA sequences – taxonomy – zooplankton.

INTRODUCTION

Investigations into the evolutionary history of daphniids have been constrained by the lack of phylogenetically informative characters. Past failures to decisively elucidate relationships among its 150 or so species have been attributed to the prevalence of both phenotypic plasticity and hybridization (Benzie, 1988a). These factors have blurred taxonomic boundaries while disguising the existence of sibling species (Taylor, Finston & Hebert, 1998; Giessler, Mader & Schwenk, 1999). In addition, the conservation of gross morphological features and the frequent convergence of characters have confused the taxonomic status of older lineages, even those partitioned into different genera (Fryer, 1991a; Colbourne, Hebert & Taylor, 1997). Although the development of a systematic classification of the group has a controversial history (Korovchinsky, 1997), few situations have been more

*Corresponding author. E-mail: jcolbour@cgb.indiana.edu

volatile than the division of species assigned to the genera *Daphnia* (Müller) and *Daphniopsis* (Sars).

Sars (1903) erected the genus *Daphniopsis* following his description of an Asian taxon (*Daphniopsis tibetana*) that appeared to be intermediate to *Simocephalus* (Schödler) and *Daphnia*. Although the number of species assigned to the genus has now grown to 11, its validity remains uncertain because some researchers believe that a few or all of its members (including the type species) are better accommodated within the genus *Daphnia* (Wagler, 1936; Hrbácek, 1987; Fryer, 1991a). Other researchers who support the validity of the genus *Daphniopsis* have recognized that it is more closely allied to *Daphnia* than to *Simocephalus* (Rühe, 1914; Hann, 1986). Finally, based on their description of the sole species known from the northern hemisphere (*Daphniopsis ephemeralis*), Schwartz & Hebert (1984) suggested that *Daphniopsis* might be ancestral to both genera. This level of disagreement makes it apparent that further efforts to resolve the affinities of deep evolutionary lineages among daphniids through morphological analyses will likely fail to produce a conclusive result.

Phylogenetic studies utilizing nucleotide variation within mitochondrial genes have had better success in delineating taxonomic relationships within the group. Analysis of sequence diversity in the 12S rRNA gene showed that the 33 species of *Daphnia* inhabiting North America include members of three deeply divergent lineages that were each assigned subgeneric status: *Daphnia*, *Hyalodaphnia*, and the ancestral *Ctenodaphnia* (Colbourne & Hebert, 1996). These subgenera show sufficient sequence divergence to suggest that they originated during the Mesozoic, approximately doubling the previous age estimate for the genus based on fossil remains (Fryer, 1991b; Smirnov, 1992). This study also suggested a close relationship between *D. ephemeralis* and species in the subgenus *Ctenodaphnia*, and this finding further disrupts the phyletic integrity of its genus. Unfortunately, the North American fauna is poorly represented by species of both *Daphniopsis* and subgenus *Ctenodaphnia* because they are more diverse on the southern continents. There is a need to extend this initial study of the evolutionary relationships among *Daphnia* by including taxa from other geographical regions.

Recently, the taxonomy of the entire Australian daphniid fauna has been revised (P. D. N. Hebert, unpubl. data), allowing for a more thorough evaluation of the systematics of the genus. Daphniids from this continent include 17 species of subgenus *Ctenodaphnia*, many of which belong to the *Daphnia carinata* (King) complex (Hebert, 1977; Benzie, 1988a, b, c). These taxa, which were once regarded as a single highly variable species (Sars, 1914), dominate ephemeral habitats in Australia. However, allozyme studies have shown that the complex is not a syngameon but, instead, is composed of at least nine closely allied species that show characteristics similar to those of the dominant species complex (*Daphnia pulex* Leydig) in North America (Hebert & Wilson, 1994). These attributes include breeding system transitions from cyclical to obligate parthenogenesis, polyploidization, abrupt genotypic shifts among local populations, and a high incidence of interspecific hybrids in some regions. Other Australian ctenodaphniid lineages (e.g. *Daphnia citrina* Hebert, *Daphnia neocitrina* Hebert) have unclear affinities (P. D. N. Hebert, unpubl. data), yet are likely to be distantly related to the rest of the fauna because morphologically similar species (*Daphnia gibba* Methuen) are found on other southern continents suggesting that they diverged prior to the breakup of Gondwana.

The Australian fauna also includes two species (*Daphnia occidentalis* Benzie, *Daphnia jollyi* Petrovski) placed within the subgenus *Daphnia* (Benzie, 1986, 1988b; Benzie & Bayly, 1996), while the saline lakes in the arid regions of the continent are dominated by six endemic species of *Daphniopsis* (Hebert & Wilson, 2000). Although Fryer (1991a) suggested that the invasion of these harsh environments has led to the radiation of this group, *Daphniopsis* is not the only daphniid to have adapted to life in salt-lakes. Three ctenodaphniids, one from North America (*Daphnia salina* Hebert & Finston) and two from the *D. carinata* complex (*Daphnia salinifera* Hebert, *Daphnia neosalinifera* Hebert), occur in lakes with salt concentrations greater than seawater. Therefore, habitat shifts into saline habitats have likely occurred on multiple occasions and each has been linked to a dramatic acceleration of molecular evolution (Hebert *et al*., 2002). Whether this rate difference is attributable to the mutagenic effect of high salt concentrations, to higher levels of damaging ultraviolet radiation in saline waters, or to some other external factor is unknown. However, a refined phylogeny of the genus, exposing the number of independent habitat transitions with associated phenotypic responses, can help dissect the environmental component affecting the diversification of daphniids.

The present study aims to resolve the systematic relationships among species of *Daphnia* and *Daphniopsis* by expanding the study to include taxa from both Australia and North America. The conclusions made with respect to the taxonomic status of ancestral daphniid lineages are based upon the relative placement of their component species onto phylogenetic trees derived from sequence diversity in three mitochondrial genes: 12S rDNA, 16S rDNA and cytochrome oxidase subunit *I* (CO *I*).

MATERIAL AND METHODS

SAMPLING AND DNA SEQUENCING

Isolates were obtained from all described species of *Daphnia* and *Daphniopsis* known to occur in Australia barring a single species, which is a recent invader (Benzie & Hodges, 1996). Seven species identified by allozyme electrophoresis but have yet to be formally described and deserving a more thorough taxonomic study were also included (Table 1). DNA was extracted from individuals that were either cultured in the laboratory, ethanol-preserved, or cryopreserved in the field, following their taxonomic assignment based on morphological and allozyme analyses. Their collection sites are listed in Table 1. Although the present study examines species from habitats in three large geographical regions (the eastern half of Australia, its south-western coast, Tasmania), there are likely to be

Table 1. The *Daphnia* species included in the present study and their collection sites

Species	Collection site
Daphnia, subgenus *Ctenodaphnia*	
carinata group	
Daphnia angulata (Hebert)	Lake Omeo, Victoria, Australia
	AY921460; AY921414; AY921453
Daphnia carinata (King)	Tasmania and Maitland, New South Wales, Australia
	AY921461; AF217116; AY921435
Daphnia cephalata (King)	Sydney, New South Wales, Australia
	AF217135; AF308967; AY921427
Daphnia longicephala (Hebert)	Wave, Western Australia and Ivanhoe, New South Wales, Australia
	AF217136; AF217114; AY921426
Daphnia magniceps (Sars)	Hoskin, Australian Capital Territory
	AF217142; AF217117; AY921433
Daphnia muddensis (new species)	Mt. Magnet, Western Australia
	AY921462; AY921415; AY921447
Daphnia nivalis (Hebert)	Lake Cootapatamba, New South Wales, Australia
	AF217143; AF217118; AY921448
Daphnia projecta (Hebert)	Nyngan, New South Wales, Australia
	AF217134; AF308966; AY921434
Daphnia reflexa (new species)	Mugga, Australian Capital Territory
	AF217133; AF308968; AY921428
Daphnia thomsoni (Sars)	Tasmania and Bombala, Victoria, Australia
	AF217144; AF217119; AY921450
Daphnia neosalinifera (new species)	Colac, Victoria, Australia
	AF217132; AY921416; AY921429
Daphnia salinifera (new species)	Lake Wyora, Queensland, Australia
	AF217131; AF217113; AY921430
Others	
Daphnia citrina (new species)	Coast, Western Australia
	AY921463; AY921419, AY921432
Daphnia exilis (Herrick)	Pond near Amarillo, Texas, USA
	AY921465; AF308972; AY921456
Daphnia lumholtzi (Sars)	Lyell Lake, New South Wales, Australia
	AY921466; AY921417; AY921451
Daphnia magna (Straus)	Crescent Lake, Nebraska, USA
	AY921467; AF217106; AY921452
Daphnia neocitrina (new species)	Mt. Magnet, Western Australia
	AY921464; AY921420; AY921431
Daphnia salina (Hebert & Finston)	Shoe Lake, Saskatchewan, Canada
	AY921469; AF308973; AY921436
Daphnia similis (Hebert & Finston)	Soap Lake, Washington, USA
	AY921468; AF308971; AY921446
Daphnia similis (Claus)	Lake in Golan Heights, Israel
	AY921470; AY921418; AY921455

Table 1. *Continued*

Species	Collection site
Daphnia, subgenus *Daphnia*	
Daphnia ambigua (Scourfield)	Little Presa, Mexico AF523716; AF523687; AF064188
Daphnia jollyi (Petkovski)[a]	Pond near Mt. Hampton, Western Australia AY921471; AF308969; AY921449
Daphnia pulex (North American lineage)	Pond near Windsor, Ontario, Canada Af117817
Daphnia occidentalis (Benzie)[a]	Northcliff, Western Australia AY921472; AY921424; AY921457
Daphnia, subgenus *Hyalodaphnia*	
Daphnia dubia (Herrick)	Van Buren County, Michigan and Wren Lake, Ontario AF064173; AY921411; AF064181
Daphnia longiremis (Sars)	Lake on Melville Penninsula, Nunavut, Canada AY921457; AY921413; AY921454
Daphnia mendotae (Birge)	Center Lake, Indiana, USA AF064174; AY921412; AY921425
Daphniopsis	
Daphniopsis australis (Sergeev & Williams)	Colac, Victoria, Australia AF217122; AF217110; AY921441
Daphniopsis ephemeralis (Schwartz & Hebert)[b]	Pond near Guelph, Ontario, Canada AY921473; AY921422; AY921439
Daphniopsis quadrangula (Sergeev)	Colac, Victoria, Australia AF217120; AF217108; AY921444
Daphniopsis queenslandensis (Sergeev)	Lake Wyara, Queensland, Australia AF217121; AF217109; AY921440
Daphniopsis pusilla (Serventy)	Rottnest Isld, Western Australia AF217124; AF217112; AY921442
Daphniopsis studeri (Rühe)	Lake Barkell, Antarctica AY921474; AY921423; AY921438
Daphniopsis tibetana (Sars)	Lake in Tibet AY921475; AY921421; AY921437
Daphniopsis truncata (Hebert & Wilson)	Coast, Western Australia AF217125; AF308965; AY921443
Daphniopsis wardi (Hebert & Wilson)	Lake Preston, Western Australia AF217123; AF217111; AY921445
Scapholebris (Schödler)	Pond near Guelph, Ontario, Canada AY921476; AY921411; AY921458

GenBank accession numbers are listed: 12S, CO *I*, 16S. GenBank ID labelled AF are archived sequences associated with earlier studies. New species have not yet been formally described by P. D. N. Hebert.
[a]Orphan taxa whose phylogenetic position is disputed[b] were previously assigned to the subgenus *Ctenodaphnia* by Colbourne & Hebert (1996).

other cryptic species in regions that are excluded from our analysis. However, we expect that such species will be closely affiliated with members of the identified fauna.

To test for the monophyletic origins of the genus *Daphniopsis* and the subgenus *Ctenodaphnia*, several taxa from other continents were also analysed. The type species of *Daphniopsis* from Asia (*D. tibetana*) and the only known daphniid from Antarctica (*Daphniopsis studeri* Rühe) were included in the present study. All of the North American and one Middle Eastern/European species of subgenus *Ctenodaphnia*, as well as selected members of the subgenera *Daphnia* and *Hyalodaphnia*, were used as references. A member of the genus *Scapholeberis* (Schödler) was used to root our phylogenetic trees because it appears to be the genus most closely related to *Daphnia* and possibly *Daphniopsis* (Taylor, Crease & Brown, 1999; Swain & Taylor, 2003).

DNA was extracted from specimens using either the Isoquick kit (Orca Research) or by boiling individuals in 6% Chelex-100 (Bio-Rad). The protocol and primer

sequences for polymerase chain reaction amplifications have been described previously (Taylor *et al.*, 1998; Havel, Colbourne & Hebert, 2001). The products were purified from agarose gels. Both DNA strands were directly sequenced using the ABI Prism TaqFS dye terminator kit (Perkin-Elmer) and electrophoresis was conducted on ABI 371 and ABI 377 sequencers.

Sequence analysis

Alignments of the rDNA sequences were first produced using ClustalW (Thompson, Higgins & Gibson, 1994). Major adjustments were then made using the DCSE editor (De Rijk & De Wachter, 1993), according to the conserved secondary structure models of arthropod (Van de Peer *et al*., 1999) and of *Daphnia* (Taylor *et al.*, 1998; Crease, 1999) rRNA molecules. Regions where the position of nucleotide insertions or deletions was uncertain were deleted from the data matrix. As a result, the 12S rDNA sequence data were reduced from an average of 562 sequenced nucleotides (ranging 559–568) to 537 aligned characters, of which 288 were variable and 249 of these were informative in cladistic analyses. Similarly, the 16S rDNA sequences (ranging 488–494 bp), were reduced to 489 aligned characters of which 211 characters were variable and 166 were cladistically informative. The 646-bp sequences for CO *I* were aligned following translation of codons to amino acids. No insertion or deletion of characters was required. The CO *I* sequence data contained 280 variable nucleotide characters, of which 265 were cladistically informative. Of the 215 amino acid characters, 27 were variable. The nucleotide and gap frequencies, including pairwise comparisons in the number of transitions and transversions, were calculated using Mega v2.1 (Kumar *et al*., 2001).

On the one hand, shared nucleotide composition bias among unrelated sequences, resulting from different processes of nucleotide substitution, can impede the accuracy of phylogenetic inferences and the test of evolutionary hypotheses (Galtier & Gouy, 1995; Tarrío, Rodríguez-Trelles & Ayala, 2001). On the other hand, inherited similarities in nucleotide composition can falsely reinforce confidence in parsimony trees (Swofford *et al*., 2001). Genes with deviant patterns of change were identified by applying a chi-square, goodness-of-fit test on the base frequencies of each sequence as implemented in PAUP* 4.0 (Swofford, 2003). Failure to account for substitution rate differences among sites can also detrimentally affect phylogenetic inferences (Yang, 1996), underestimate branch lengths (Buckley, Simon & Chambers, 2001), compromise the power of likelihood ratio tests (Zhang, 1999), and exacerbate compositional bias problems (Conant & Lewis, 2001). Estimates of the gamma shape (α) (whose value is comparable across datasets and inversely related to the magnitude of rate variation) among other parameters best describing the molecular evolutionary model were obtained using TREE-PUZZLE, version 4.0.2 (Strimmer & von Haeseler, 1997). The model that best describes the process of nucleotide substitution for the combined dataset was identified by the Akaike Information Criterion (AIC; Akaike, 1974), as executed by MODELTEST, version 3.06 (Posada & Crandall, 1998). Site-specific rates models were not investigated because they explicitly assume rate homogeneity within each rate class and can mislead estimates of tree topology (Buckley, Simon & Chambers, 2001); molecular evolutionary rates are known to vary across the genus *Daphnia* (Hebert *et al*., 2002).

Phylogenetic analyses

Five partitions were created for exploring the sequence data. Three partitions consisted of sequence matrices for each of the genes. Genes were subsequently combined to form the fourth (12S with 16S) and fifth (12S, 16S, CO *I*) partitions. The relative content of their phylogenetic signals was evaluated in two ways. First, skewness test statistics were obtained (g1; Hillis & Huelsenbeck, 1992) by performing searches of five or 15 taxa drawn according to a structured random selection. This taxonomic sampling design aimed to determine the appropriate combination of data to resolve both shallow and deeply divergent clades under maximum parsimony optimality criteria. Sampling sets of five involved members either of the *D. carinata* complex, *Daphniopsis* or subgenus *Ctenodaphnia*, with the a priori assumption that each of these groups is monophyletic. Sets of 15 consisted of taxa randomly chosen from among members of the genus *Daphnia*. Two species, *D. jollyi* and *D. occidentalis*, whose taxonomic placement is uncertain, were excluded from these analyses. Each set was reconstructed and tested ten times, to enable the calculation of summary statistics. Second, for an evaluation of phylogenetic quality of each partition under a maximum likelihood optimality criterion, likelihood-mapping was performed (Strimmer & von Haeseler, 1997). This method computes and summarizes the probabilities of obtaining fully resolved phylogenies for each possible quartet of sequences belonging to a priori defined groups, except for the genus *Daphnia*, whose assessments are based on random samples of 1000 quartets. An ideal dataset for phylogenetic analyses would include all characters and simultaneously provide signal at the base and tips of the phylogenetic tree without the addition of noise.

Optimal tree topologies were investigated using two selection criteria. First, a cladistic analysis of the nucleotide characters was performed using maximum

parsimony (MP) in PAUP*. No constraint on character state changes was imposed, but gaps (indels) in the sequence alignment were coded as missing characters because of uncertainties in modelling the number of events leading to multiple insertions and deletions. Noise obscuring the phylogenetic signal (homoplasy) was reported by the consistency (CI) and retention (RI) indices. Confidence in clades was assessed by calculating the jackknife monophyly index (JMI; Siddall, 1995a, b) and by evaluating the decay index (DI; Bremer, 1994) using AutoDecay, version 2.9.8 (Eriksson, 1995). The jackkife results were reported as the proportion of pseudo-replicated parsimonious trees that validate each grouping following the removal of each taxon. The decay index showed support for a monophyletic group by calculating the difference in tree length between the shortest trees with and without that group. Second, a Bayesian phylogenetic method based on the likelihood function was also applied to the data using MRBAYES, version 2.01 (Huelsenbeck & Ronquist, 2001) for its ability to better accommodate complex models, which can include unequal nucleotide frequencies, variation in the substitution rates among sites, and branch length heterogeneity, by approximating their posterior probabilities (Huelsenbeck *et al*., 2002).

Phylogenetic hypothesis testing

A priori hypotheses relating to the monophyly of the *D. carinata* complex, of the genus *Daphniopsis* and of the single origin to the subgenus *Ctenodaphnia* were evaluated using posterior probabilities for sequence matrices of each gene and for the combined data. This test allowed an efficient statistical evaluation of phylogenetic evidence for monophyly, while examining congruence among datasets.

Measuring habitat specific rates of molecular evolution

Relative rate tests were performed between phylogenetic lineages inhabiting freshwater, saline and high ultraviolet (UV) environments by the method proposed by Li & Bousquet (1992), which samples one or more taxa per lineage and circumvents statistical problems linked to non-independent comparisons. Results were obtained using RRTree, version 1.1.11 (Robinson *et al*., 1998; Robinson-Rechavi & Huchon, 2000).

RESULTS

Sequence diversity

Sequence comparisons at both 12S and 16S ribosomal genes among the 36 ingroup taxa show remarkable nucleotide variation; the Kimura (1980) corrected pairwise divergence estimates extend from 0.2% to 27% overall. Comparisons between members of the *D. carinata* complex yield estimates under 11%, whereas sequence divergences for all ctenodaphniids do not exceed 18% (average = 10%). Interestingly, divergence values are significantly greater between *Daphniopsis* species, ranging from 10% to 19% (average = 16%). The maxima for both subgenus *Ctenodaphnia* and *Daphniopsis* are similar to the largest 12S sequence divergence measured within the three subgenera in North America (~20%), at which point, saturation of transitional substitutions within the genes becomes apparent. The high number of variable sites containing three (28%) and four (20%) nucleotides also indicates substitutional saturation within the rDNA dataset. Given this large sequence divergence among rRNA genes, there is no surprise in discovering greater saturation among synonymous sites within CO *I* sequences, which diverge from 1% to 31% (average = 23%). All third codon positions are variable, while 49% contain four nucleotides. Because the removal of saturated sites from the dataset decreases the phylogenetic signal when multiple character state changes have occurred over an extended period of time (Philippe *et al*., 1996; Yang, 1998; Broughton *et al*., 2000), no variable characters are excluded from subsequent analyses.

Compositional variation is evident within all sequenced fragments. Both rRNA genes are A-T rich, with an average content of 65% and 68%. The A-T content of CO *I* is also elevated (59%). Yet, unlike in rDNA, thymine and adenine are not evenly represented; thymine exceeds all other nucleotides by contributing 36% to the total composition. This nucleotide bias is more pronounced when disregarding invariable and uninformative sites, which then bolsters thymine's fraction to 39% within 12S and to 44% within CO *I*. Accordingly, 12S and CO *I* datasets fail the chi-square test of nucleotide homogeneity when reduced to parsimony sites, indicating significant deviations from stationary among species. Such deviations are apparent within CO *I* when comparing subgenus *Ctenodaphnia* and *Daphniopsis* species, which differ in average thymine content by 5% and in average cytosine content by 4%. However, greater differences are observed between species within a single group. For example, *Daphnia angulata* possesses the lowest cytosine content (10%) among the subgenus *Ctenodaphnia*, whereas *D. salina* has the highest content at 20%. Despite the apparent homoplasy and compositional bias within the data, a priori tests of phylogenetic signal produce positive results.

Phylogenetic signal within partitioned data

Of the five partitions, the combined 12S and 16S datasets consistently reveal the most phylogenetic

signal under maximum parsimony optimality criteria, by scoring the best average skewness test value (g1) (Hillis & Huelsenbeck, 1992) within each taxonomic grouping, save the *carinata* group where g1 is insignificantly elevated by the addition of 16S data (Table 2). Overall, g1 values increase with further addition of CO *I* data for all groups, despite augmenting the number of characters by 47–62%. In one clear case (*carinata* group; Table 2), the total data contain, on average, less phylogenetic structure than the 12+16S partition, dropping its significance level from 0.01 to 0.05 compared to random data. Although this preliminary assessment of data quality for parsimony analyses is intentionally conservative, as a result of restricting the number of taxa examined to be small (five for three groups) while increasing the number of characters (Hillis & Huelsenbeck, 1992), the relative value of each partition remains; character sets derived from rDNA sequences are more informative than those obtained from CO *I*. However, all partitions hold significant phylogenetic signal for parsimony analyses when less restrictive numbers of taxa are included.

By contrast, CO *I* and rDNA sequences are equally informative under maximum likelihood, by scoring matching numbers of resolved quartets within *Daphniopsis*, *Ctenodaphnia*, and *Daphnia* groups (Table 3). Yet within the *carinata* group, CO *I* data contain the greatest phylogenetic content, resolving over 94% of all possible quartets. The relatively consistent information content of all loci is attributed to the method's ability to better accommodate large variations in the substitution rates among sites. Rate heterogeneity estimates based on gamma parameter values range from extreme ($\alpha = 0.02 \pm 0.26$; standard error obtained by the curvature method) for 16S rDNA to weak heterogeneity ($\alpha = 2.74 \pm 1.26$) for CO *I* data within the *carinata* group. Although the rate variation among partitions was less severe for data sampled from the whole genus ($\alpha = 0.26 \pm 0.02$ to 0.50 ± 0.03), the among-site heterogeneity is sufficiently strong to negatively impact phylogenetic reconstruction and bias estimates of evolutionary distances under simple, less-realistic, models of molecular evolution (Yang, Goldman & Friday, 1994). Nevertheless, the analysis indicates that the total data should provide maximal phylogenetic signal under likelihood optimality criteria.

Cladistic trees based on total sequence divergence

Maximum parsimony analysis of the total data produces a single most parsimonious tree, which reveals that all Australian daphniids are historically linked within the subgenus *Ctenodaphnia* (Fig. 1) and that the fauna is comprised of five distinct lineages. Group 1 consists of every member of the *D. carinata* complex and includes *D. jollyi*, a species previously believed to represent an ancient lineage within the subgenus *Daphnia*. These 13 species are appropriately classified as forming a species complex (*sensu* Colbourne & Hebert, 1996), for at least five species regularly produce interspecific hybrids in nature (Hebert & Wilson, 1994) and the maximum sequence divergence at 12S between all species is 14%, which marginally fits the divergence criterion of 14% at 12S used to delineate species complexes within the North American fauna. Group 2 consists of a single species (*Daphnia lumholtzi* Sars) that is distantly allied to *Daphnia similis* (Claus) from Israel and *Daphnia magna* (Straus) found in North America, suggesting that *D. lumholtzi* is an invader of Australia that has evolved independently from other populations found in Africa and Asia. Group 3 contains a pair of related species inhabiting western Australia that cluster with *D. salina* from North America. *Daphnia citrina* and *D. neocitrina* show 9% sequence divergence at 12S and are amply divergent from *D. salina* (Hebert & Finston) NA (20%) to be classified within a separate *D. citrina* species complex. Group 4 contains only members of the genus *Daphniopsis*; species that are restricted to Australia cluster together, while the two congeners found on other southern continents stem from the base of the lineage. Therefore, we propose that all *Daphniopsis* be reassigned to the genus *Daphnia*. However, these taxa show an average sequence divergence equivalent to genetic distances that bound subgeneric relationships within North America. Thus, the group may also merit subgeneric status, depending on the results of the Bayesian analyses (see below). Nevertheless, the parsimony tree suggests that *D. ephemeralis* NA is distantly related to *Daphniopsis*. Finally, Group 5 is represented by a single taxon, *D. occidentalis.* This species, which was previously assigned to the subgenus *Daphnia*, is instead identified as the most genetically divergent daphniid on the continent.

Support for clades

The *D. carinata* species complex is a strongly supported monophyletic group with weak internal structure. The clade is distinct from *Ctenodaphnia* species inhabiting other continents (JMI = 97; DI = 9) and its basal group is likely composed of three closely-related species [*reflexa* (*projecta*, *cephalata*)]. Other supported groupings within the *D. carinata* complex include *Daphnia nivalis* (Hebert) with *Daphniopsis thomsoni* (Sars), *Daphnia muddensis* (Hebert) with *Daphnia longicephala* (Hebert) and *D. salinifera* with *D. neosalinifera*. Additional branch-and-bound searches for resolving relationships within the

Table 2. The relative content of phylogenetic signal within five partitions of the total data, measured by plotting 100 000 trees drawn at random from all possible topologies as a function of tree length to obtain the g1 kurtosis statistic

	12S g1	12S # char	16S g1	16S # char	CO *I* g1	CO *I* # char	12+16S g1	12+16S # char	Total g1	Total # char
carinata group										
Average	–1.07**	27.3	–0.81	11.5	–0.72	62.1	–1.02**	38.8	–0.85*	100.9
Minimum	–1.37	15	–1.37	9	–1.04	48	–1.31	24	–1.17	75
Maximum	–0.38	37	–0.13	14	–0.26	78	–0.12	51	–0.17	124
Number significant	9	4	6	8	7					
Daphniopsis										
Average	–0.46	64.9	–0.64	37.4	–0.09	106	–0.68	102.3	–0.58	208.3
Minimum	–1.14	57	–1.29	33	–0.51	85	–1.23	91	–1.2	177
Maximum	0.38	72	–0.04	45	0.3	119	–0.24	112	0.26	231
Number significant	2	5	0	3	4					
Ctenodaphnia										
Average	–0.54	32.1	–0.56	17.8	–0.35	78.8	–0.71	49.9	–0.55	128.7
Minimum	–1.29	19	–1.16	7	–0.99	67	–1.32	26	–1.08	94
Maximum	0.22	43	0.08	25	0.31	100	–0.04	63	–0.08	161
Number significant	3	3	3	5	3					
Genus *Daphnia*										
Average	–0.81**	162.7	–0.69**	99.8	–0.44**	231.9	–0.84**	262.5	–0.67**	494.4
Minimum	–1	141	–1.05	88	–0.79	221	–1.16	229	–0.78	450
Maximum	–0.61	179	–0.44	109	–0.27	238	–0.65	285	–0.55	519
Number significant	10	10	10	10	10					

The results are summarized from ten replicates of taxon sampling within each a priori defined group. Data partitions within groupings that produce more negative g1 values are more phylogenetically informative. The number of parsimony characters is shown (# char). Datasets that contain, on average, significantly more structure than random data are marked by asterisks (*$P < 0.05$ and **$P < 0.01$). The number of significantly structured datasets from a sample of ten is also shown (# significant).

CO *I*, cytochrome oxidase subunit *I*.

Table 3. Phylogenetic content within five partitions of the total data, measured by likelihood-mapping (LM)

	carinata group			*Daphniopsis*			*Ctenodaphnia*			Genus *Daphnia*		
	ΣA_i	A^*	ΣA_{ij}	ΣA_i	A^*	ΣA_{ij}	ΣA_i	A^*	ΣA_{ij}	ΣA_i	A^*	ΣA_{ij}
12S: 537 bp	68.3	26.7	5.0	79.3	14.3	6.4	78.1	15.5	6.4	77.7	15.6	6.7
16S: 490 bp	76.2	17.0	6.8	79.3	6.3	14.4	70.7	22.0	7.3	73.3	17.9	8.8
CO *I*: 646 bp	94.5	1.6	3.9	77.7	5.6	16.7	77.7	7.6	14.7	73.8	7.5	18.7
12+16S: 1027 bp	79.2	17.2	3.6	83.3	7.9	8.8	82.7	11.0	6.3	85.4	8.9	5.7
Total: 1673 bp	93.1	4.2	2.7	83.4	2.4	14.2	90.4	5.3	4.3	90.4	3.6	6.0

Results are summarized following Strimmer & von Haeseler (1997). Shown for each a priori defined group are: (ΣA_i) cumulative percentages for fully resolved topologies of quartets mapped into the tree-like regions (A_1, A_2, A_3) of a LM triangle; (A^*) percentages of quartets forming star-like, unresolved, phylogenies; (ΣA_{ij}) percentages of quartets forming phylogenies that are not completely resolved and falling within the net-like regions (A_{12}, A_{13}, A_{23}) of a LM triangle. Selection of the molecular model was based on prior results obtained using MODELTEST, version 3.06 (Posada & Crandall, 1998), suggesting that parameter-rich models best describe the data. The Hasegawa–Kishiro–Yano (HKY) molecular model was employed with rate heterogeneity by estimating one invariable and four gamma rate parameters from the data. Nucleotide frequencies and the transition/transversion parameter were also estimated from the dataset.
CO *I*, cytochrome oxidase subunit *I*.

complex by using functional outgroups produce only some new insights because the topologies vary according to the choice of an outgroup (trees not shown): *D. carinata* is most often linked to *D. jollyi* and numerous trees propose that (*angulata*, *magniceps*) is a sister-group to (*nivalis*, *thomsoni*) as an alternative arrangement to Figure 1. These uncertainties about relationships within the complex are reflected by the minimal level of support at four internal branches (DI = 1–2); their dislocations produce equally parsimonious trees with one to two extra steps to the overall tree length.

The most parsimonious tree reinforces the historical ties of *D. lumholtzi* to *Daphnia* from other continents and provides modest cladistic support (DI = 3) for its connection to *D. similis* from Israel and *D. magna* NA. This tree also indicates that the conventional groupings *Ctenodaphnia* and *Daphniopsis* are reciprocally paraphyletic. The *Daphniopsis* clade is clearly separate from *D. ephemeralis* NA, which is positioned at the base of the ctenodaphniids. These, in turn, are split by the placement of a *Daphniopsis* clade interior to [*salina* (*citrina*, *neocitrina*)]. Nonetheless, the data authenticate Australia's endemic radiation of *Daphniopsis* (JMI = 100; DI = 5) while denoting its close affiliation to species found in Asia and Antarctica. There are no alternative topologies suggested by analyses of the total data, and only slight rearrangements within the *D. carinata* complex are observed by analyses of the combined ribosomal genes (tree length = 2222; CI = 0.34; RI = 45). But confidence indices at the basal nodes of the subgenus *Ctenodaphnia* are poor, and analyses restricted to 12S characters produce six equally parsimonious trees (length = 1369; CI = 0.33; RI = 0.45) that either place *D. ephemeralis* ancestrally to all *Daphniopsis* (five of six trees) or at the root of the Australian radiation. Furthermore, all six trees include a single ctenodaphniid (*D. salina*) within *Daphniopsis* while elevating the group to a more derived position within the subgenus. By contrast, 31 of 249 equally parsimonious trees restricted to 16S characters (length = 831; CI = 0.36; RI = 0.47) support the monophyly of subgenus *Ctenodaphnia* (albeit breaking other subgeneric groupings). Such drastic shifts in the ordering of deep branches likely result from homoplasy within sequences that have attained transitional saturation, compounded by long-branch attraction among some ancient taxa.

Using *Scapholebris* to root the phylogenies, *D. occidentalis* is placed outside of the genus *Daphnia*. Because the addition of only two steps to the tree length consigns this species to *Daphniopsis* and subgenus *Ctenodaphnia*, there is little assurance for its present phylogenetic position. However, cladistic analysis does reject its inclusion within the subgenus *Daphnia* because 25 extra steps are then added to the length of the parsimony tree.

Bayesian analyses of the total data

The AIC indicates that the parameter-rich GTR + I + Γ model best fits the combined dataset (–lnL = 20634.02; AIC = 41288.03; α= 0.01), whereas optimal models for individual genes are either identical (12S) or special cases of the same model. The general time reversible (GTR) model imposes no assumption on the nucleotide frequencies, specifies six substitution rates, defines a proportion of invariant sites (I) and incorporates a

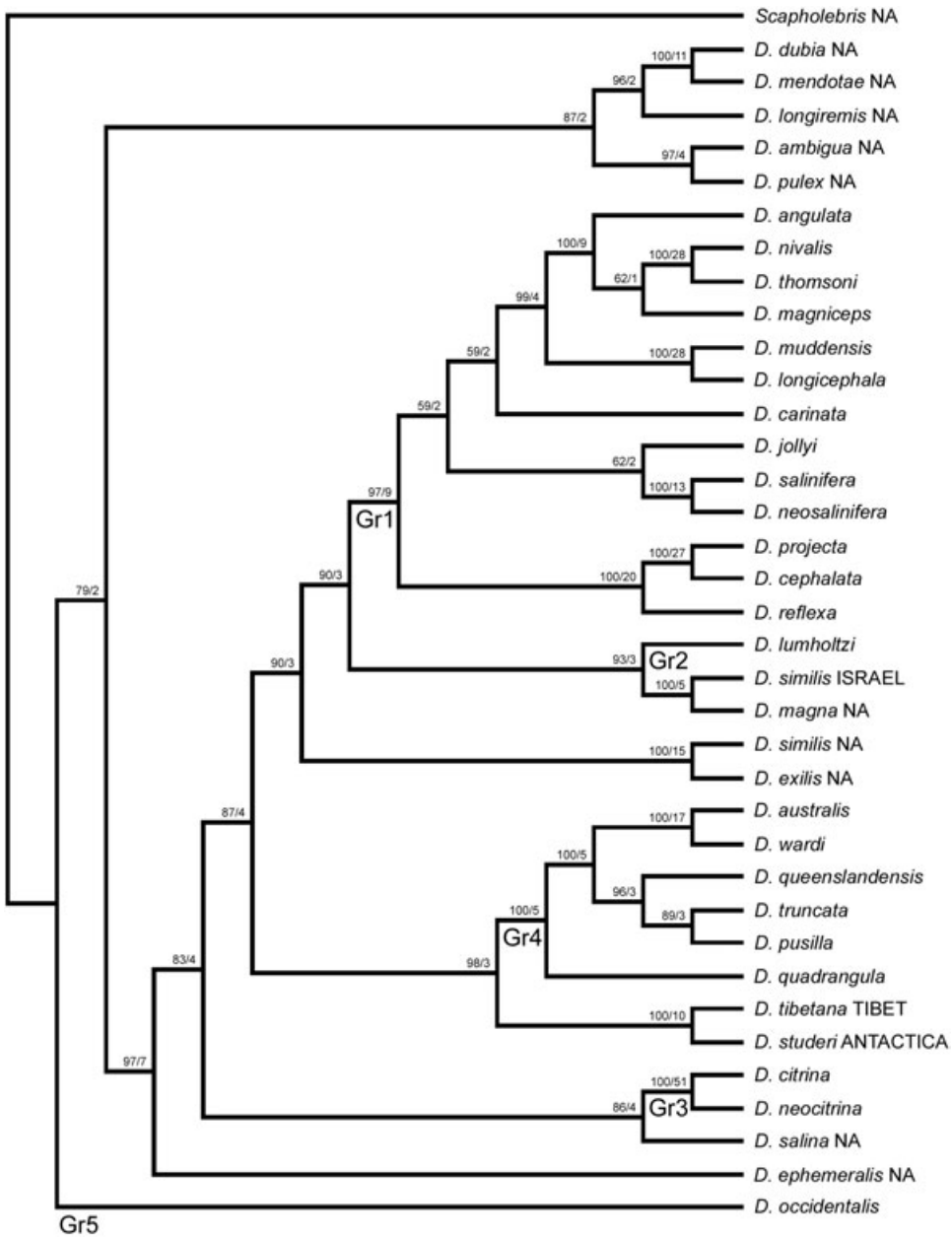

Figure 1. The single most parsimonious tree derived from the analysis of the full data (length = 4775, CI = 0.27, RI = 0.37 with 680 parsimoniously informative characters) using *Scapholebris* for an outgroup. All nucleotide characters are unordered and weighted equally. Gaps are ignored. The tree is resolved from a heuristic search; taxa are added randomly with 100 replications and with ten trees held at each step. Multrees and steepest descent options are invoked. The jackknife monophyly index followed by the decay index is shown at each node. Gr1–5 correspond to the five *Daphnia* lineages found in Australia.

gamma correction (Γ) for among-site rate variation. Phylogenies obtained by Bayesian analyses implementing these parameters support a monophyletic origin of the *D. carinata* complex that is rooted by a species ancestral to *D. salinifera* and *D. neosalinifera* (Fig. 2A). Three internodes show posterior probabilities under 90% (Fig. 2A) indicating the uncertain placement of (*carinata*, *jollyi*). The Bayesian consensus tree also links *D. lumholtzi* with *D. similis* from Israel.

A minority of trees (24%), sampled from the posterior probability distribution of interest, place (*citrina*, *neocitrina*) at the base of a *Ctenodaphnia* grouping that excludes North American *D. salina*. This poorly

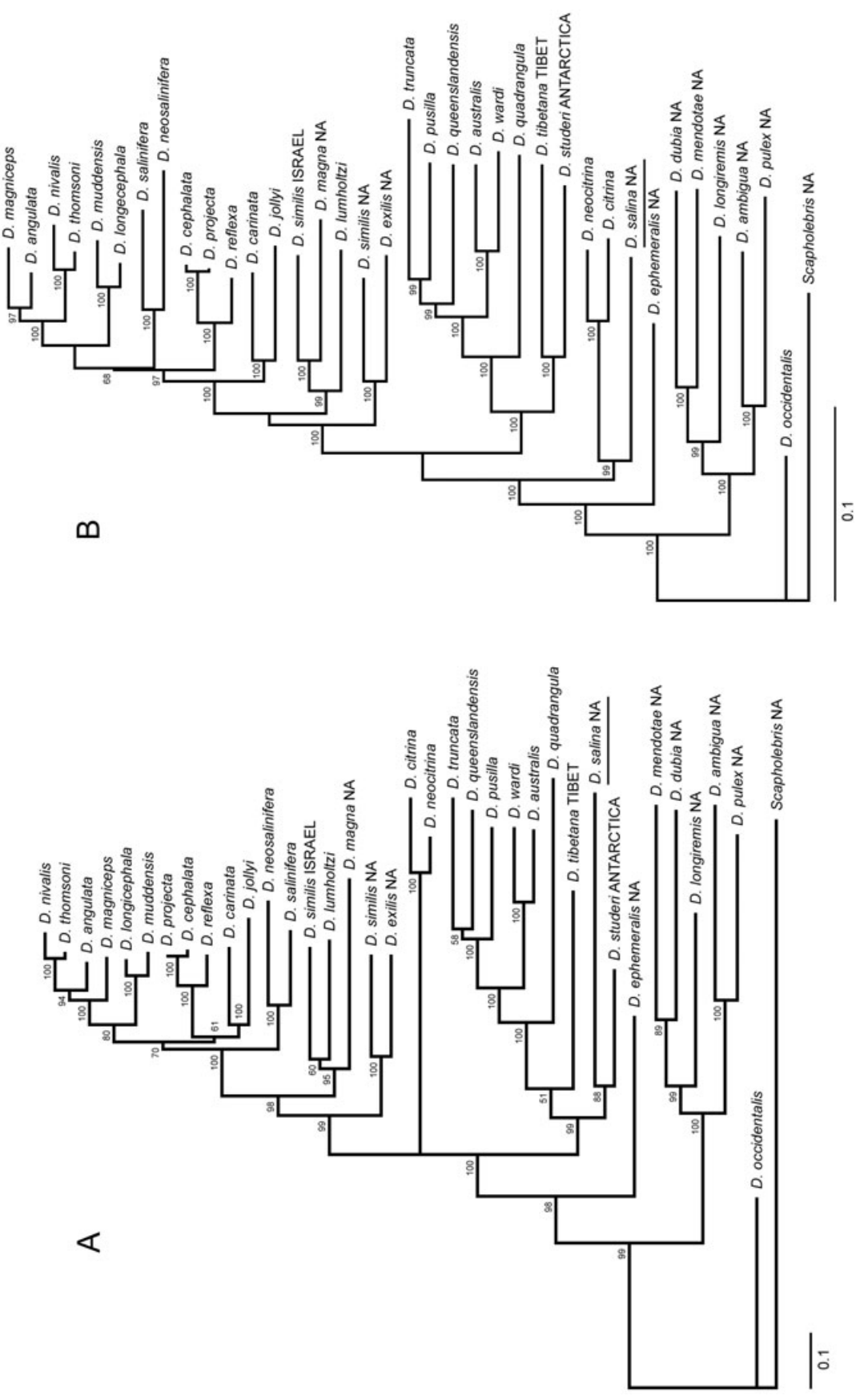

Figure 2. Trees produced by Bayesian phylogenetic analyses of the total data. Four Markov chains (three heated and one cold) are simultaneously started from a random tree and run for 10^6 generations with sampling occurring every 100 generations; 200 000 generations (20%) are discarded as 'burn-in'. Default settings are used for the priors on the rate matrix (0–100), branch lengths (0–10), proportion of invariable sites (0–1), and the Γ shape parameter (1–10). The base-frequency parameter is set to a dirichlet distribution and an uninformative prior is used. Each analysis is performed twice. A, results using a GTR + I + Γ substitution model. The best tree is sampled at generation 975 300 with a log-likelihood (lnL) score of –20 613.25. B, results using the GTR model assuming equal rates of nucleotide substitutions across sites. The best tree is sampled at generation 274 700 with a lnL score of –24 482.38. The branch-lengths reflect the amount of evolution assuming each of the models. Values at the nodes are posterior probabilities for the membership of groupings shown as percentages.

Table 4. The posterior probability (%) of presumed monophyletic groupings based on separate Bayesian analyses of the three genes and of the total data applying two models of molecular evolution, which differ by the inclusion of among-site rate variation parameters (I + Γ)

	12S		16S		CO *I*		Total data	
Hypothesis	GTR + I + Γ	GTR	GTR + I + Γ	GTR	GTR + I + Γ	GTR	GTR + I + Γ	GTR
(A) *carinata* group	97.1	100	66.7	100	44.3	100	100	100
(B) *Daphniopsis*	0	0.3	3.1	3.1	0	0	0	0
(C) *Ctenodaphnia*	1.6	0.4	0.6	39.1	0	0	0.1	0.2
(B) + (C) + *Daphniopsis occidentalis*	49.2	0.1	45.1	0.8	0	0	0.6	0.1

The probabilities are recorded as the proportion of 8000 trees (sampled after reaching stationarity) that contain the group of interest at the exclusion of other taxa.
CO *I*, cytochrome oxidase subunit *I*; GTR, general time reversible.

supported branch is collapsed, forming a polytomy in Figure 2A because 44% of the trees alternatively stem (*citina*, *neocitrina*) from the leading branch to a clade composed mostly of *Daphniopsis*. With the confident pairing of *D. salina* NA with *D. studeri* in preference to other ctenodaphniids, and with the segregation of *D. ephemeralis* to a position ancestral to *Ctenodaphnia* and *Daphniopsis*, there is apparently no molecular defense for upholding distinctions between members of these groups. This interpretation is reinforced by extremely low posterior probabilities of presumed monophyletic groupings from analyses of combined and partitioned datasets, indicating congruence among the different genes (Table 4). Effectively, no trees are observed that solely group species of *Daphniopsis*, or members of the subgenus *Ctenodaphnia*. However, although the combined data places *D. occidentalis* outside the genus *Daphnia*, almost half the phylogenies reconstructed from the ribosomal genes suggest that this taxon represents a distinct *Daphnia* lineage (Table 4).

The incongruence between the trees derived from Bayesian and molecular phylogeny (MP) analyses are reconciled when among-site rate variability (I + Γ) are constrained. Assuming equal rates of nucleotide substitutions across sites, *D. salina* is grouped once more with (*citrina*, *neocitrina*) at a deep interior node, separating *D. ephemeralis* from an otherwise monophyletic grouping of *Daphniopsis* (Fig. 2B). The similar phylogenetic patterns extend to analyses of partitioned datasets; the subgenus *Ctenodaphnia* has a single origin in nearly 40% of the sampled 16S trees (Table 4). The main exceptions are found when comparing relations among species in the *D. carinata* complex. Both models and methods produce different arrangements at its root node. Even so, there is a clear presence of rate heterogeneity in the data at greater levels of divergence, which is obscuring the phylogeny.

Testing for habitat-specific differences in evolutionary rates

The majority of phylogenetic trees identify three habitat shifts from fresh water to saline environments (Fig. 3), where both ionic and UV exposure are extreme. The ancestor to the *Daphniopsis* of southern continents is shown to be one of the earliest daphniids to have invaded saline lakes. Independent transitions were also made by *D. salina* in North America and by a single lineage within the *D. carinata* complex (*D. salinifera*, *D. neosalinifera*). Additional transitions of interest are made apparent by the evolution or maintenance of a heavily melanized carapace used to quench UV radiation in two species that occupy oligohaline habitats. *Daphnia jollyi* is restricted to shallow, soft-water, granite-rock domes in Western Australia and thus particularly susceptible to UV (Hessen & Rukke, 2000). Similar to the other members of the *D. carinata* complex, its ancestral habitat is freshwater. Quite the opposite, *D. studeri* inhabits the clear-water, UV-rich, glacial lakes of Antarctica and likely represents a species which moved from saline back to fresh water. If increased mutation rates result solely from higher UV exposure in saline environments, then relative-rate tests should indicate no significant differences among these five lineages. Yet, all should show significant rate acceleration compared with freshwater species unaffected by UV (Fig. 3).

The results obtained from these tests show an interesting pattern (Table 5), suggesting that molecular clocks deviate within all lineages exposed to UV radiation and that molecular evolutionary rates are a function of dosage specific to particular habitats. When comparisons are made among partitions within the *D. carinata* species complex, both the UV-1 and Saline-1 lineages are found to diverge significantly faster than the remaining freshwater lineage. Moreover, their substitution rates do not differ, suggesting

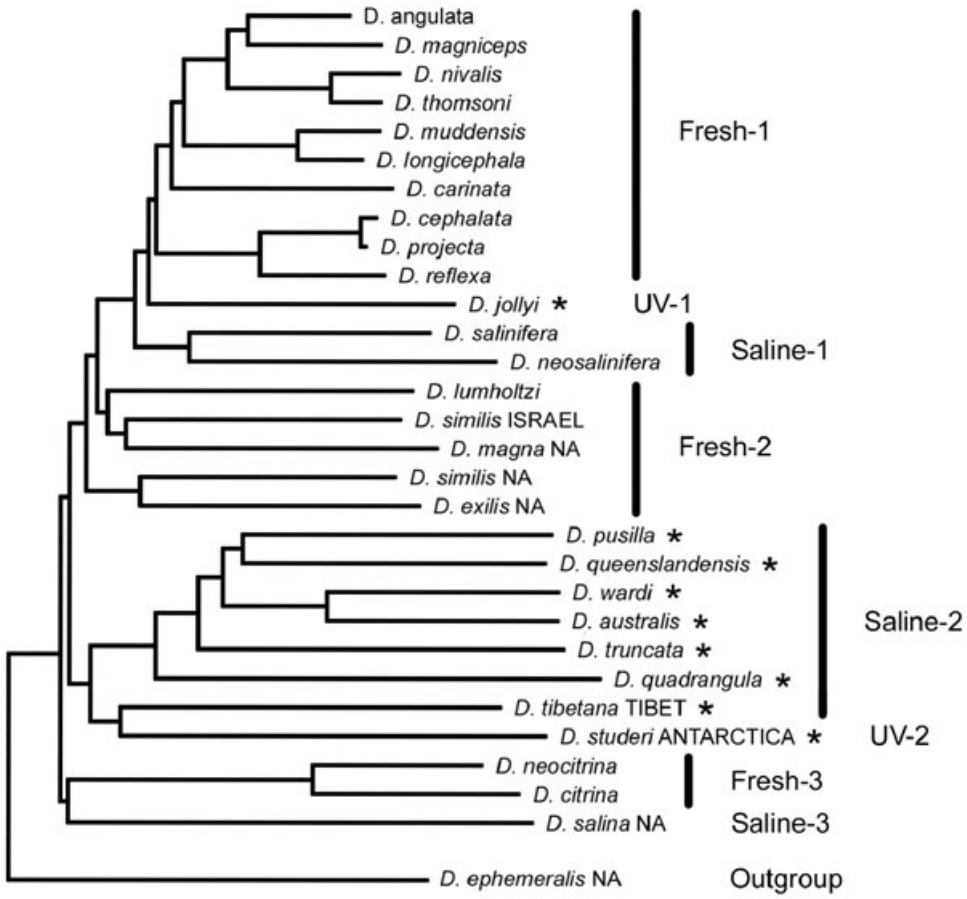

Figure 3. Lineages within the phylogenic tree indicating their habitat occupancy of fresh, saline, and ultraviolet (UV)-rich waters. The partial tree was constructed by Neighbour-joining using a Kimura two-parameter weighted distance matrix. Species with a carapace pigmented with melanin are indicated by an asterisk.

Table 5. Results of relative-rate tests among selected groups inhabiting freshwater, ultraviolet (UV)-rich, and saline environments

Partition	Fresh-1	UV-1	Saline-1	Fresh-2	Saline-2	Fresh-3	UV-2	Saline-3
Fresh-1	–	–2.80**	–4.34**					
UV-1	–1.53	–	–0.70					
Saline-1	–2.39*	–0.38	–					
Fresh-2	–0.72	0.97	1.67	–				
Saline-2	–3.27**	–1.72	–1.54	–3.01**	–			
Fresh-3	–2.69**	–1.07	–0.80	–2.32*	0.81	–		
UV-2	–2.59**	–1.25	–1.03	–2.36*	0.50	–0.27	–	
Saline-3	–4.55**	–2.35*	–2.36*	–4.59**	–0.29	–1.29	–0.92	–

The total data were used to calculate the mean distance between groups over the standard error, weighted by the two-parameter distance of Kimura (1980) and by the topology of the phylogenetic tree shown in Figure 3. *Daphnia lumholtzi* was used as the reference outgroup for comparisons among partitions of the *Daphnia carinata* complex shown in the upper matrix. *Daphnia ephemeralis* was used as the reference outgroup for all pairwise comparisons shown in the lower matrix. Significant differences in substitution rates are indicated by asterisks (*$P < 0.05$ and ** $P < 0.01$). The discrepancy between both matrixes results from using a more distant outgroup for the global comparision, which increases the variance estimates (Robinson *et al*., 1998).

that the common environmental factor (UV) is impacting evolutionary rates. Further inspection of the data also indicates that some saline lineages are more accelerated than others. For example, all saline groups have faster clocks when compared to freshwater taxa of the *D. carinata* complex (Fresh-1), yet the *Daphniopsis* group (Saline-3) has a significantly greater rate than Saline-1. This difference coincides with the ranges of salt concentration measured in lakes occupied by these two groups; conductivities vary from ~20 000 uS cm^{-1} to >100 000 uS cm^{-1} for lakes with *Daphniopsis*, yet habitats containing *D. salinifera* and *D. neosalinifera* rarely exceed 30 000 uS cm^{-1}. Although UV radiation is proportionally greater in lakes with higher salinities (Arts *et al*., 2000), levels of radiation vary as well in freshwater habitats and likely have the same modulating effect on rates of molecular divergence. For example, *D. studeri* (UV-2 in Table 5) shows no significant difference in substitution rates compared with the three saline groups. Yet, rate differences between *D. jollyi* and Saline-3 are significant, despite the rate increases in all three lineages. Finally, our data suggests that the *D. citrina* species complex (Fresh-3) also has an accelerated molecular clock. By contrast to the other lineages, this rate difference is unlikely the result of habitat-specific traits linked to UV radiation.

DISCUSSION

Resolving the status of taxonomic groups using molecular sequence data is generally straightforward. By drawing on variable nucleotide characters, phylogenetic analyses cluster species into clades that are either closely related to, or deeply divergent from, recognized members of the genus *Daphnia*. Indeed, the patterning of genetic divergence observed in the present study shows that all species of *Daphniopsis* (including the type species) are internal to *Daphnia*, and are thus properly treated as a component of this genus. The same patterning also provides insight into the series of evolutionary events that directed the diversification of the five lineages that constitute the Australian fauna. However, in contrast to earlier work on the North American (Colbourne & Hebert, 1996) and more recent work on South American (Adamowicz, Hebert & Marinone, 2004) *Daphnia* that revealed a genus sharply divided among three ancient lineages, the present survey of sequence diversity on North American and Australian continents shows that cladogenesis has been a more ongoing affair, which complicates the delineation of subgeneric boundaries.

Past molecular information about the phylogenetic relationships among representatives of the major morphological forms suggested that the genus should be partitioned into three subgenera, whose group means differed by 24–25% sequence divergence at 12S rDNA, while their constituent species never exceeded 20% (Colbourne & Hebert, 1996). Subsequent studies using other mitochondrial and nuclear genes (Schwenk, Posada & Hebert, 2000; Omilian & Taylor, 2001) confirmed the taxonomic distinction among *Daphnia*, *Hyalodaphnia*, and *Ctenodaphnia*. However, the addition of Australian and key reference taxa to this phylogenetic scaffold indicates that two other assemblages show more than 20% sequence divergence from existing groups and may warrant subgeneric status. The first divergent group includes only *D. occidentalis* (Group 5). Although this species was initially assigned to the subgenus *Daphnia* (Benzie, 1986), its nucleotides at 12S differ on average by 29%. Its recognition as a separate subgenus is reinforced by the species' unique morphology; *D. occidentalis* has distinctive abdominal processes and produces a single-egged ephippium that is otherwise known only in *Daphniopsis pusilla* (Serventy). The phylogenetic trees suggest that *D. occidentalis* originated prior to the diversification of all the other subgenera. This position is supported by a study using sequence data from the nuclear large subunit rRNA gene, which also suggested this species might truly represent the most ancestral daphniid lineage (Omilian & Taylor, 2001). Based on these results, we propose that a new subgenus, *Australodaphnia*, be recognized with *D. occidentalis* as its sole member.

The second divergent group consists of taxa originally assigned to *Daphniopsis*. The sequence data obtained from the present study convincingly indicate that all six species sampled in Australia represent an endemic radiation (Group 4), which shares a common ancestor with species found in Asia and Antarctica, but not with *D. ephemeralis* from North America. A prior morphological investigation had suggested a polyphyletic origin for the *Daphniopsis* assemblage (Hann, 1986). The present study confirms this notion. However, the large extent of sequence divergence between the two *Daphniopsis* lineages also suggests their joint origin with *Ctenodaphnia* in a brief burst of diversification during the Mesozoic, at least 100 Mya. Although Group 4 and its allies do show 21–28% sequence divergence from all other groups within the genus, difficulties in marginalizing these lineages into subgenera are evident. All phylogenetic trees derived from analyses of the total data suggest that *D. ephemeralis* is ancestral to two alternative arrangements of *Ctenodaphnia*, which are both split by the remaining species of *Daphniopsis*. One arrangement, specified by MP and Bayesian trees that ignore shifts in evolutionary rates, suggests that a deeply divergent clade roots an otherwise monophyletic group of *Ctenodaphnia* with a *Daphniopsis* lineage. The other arrangement, based on Bayesian analyses that accord

varying rates of evolution among sites, suggests that *D. salina* belongs to an otherwise distinct lineage of *Daphniopsis*. In light of this lack of phylogenetic congruence among methods, and of the existence of a growing number of deeply divergent species that share closer ties with members of other continents, no further subgenera are proposed without first including a more global sampling of taxa. Nevertheless, additions of *Daphniopsis* isolates from other regions will unlikely oppose its current standing within the genus *Daphnia*.

Our analyses all indicate that the Australian fauna is composed of three other ctenodaphniid lineages for a total of five. The *D. carinata* complex (Group 1) is shown to be the most speciose lineage on this continent, whose 13 species prevail within the intermittent ponds, particularly throughout the south-east. The extent of 12S rDNA sequence divergence within this group is sufficiently small to recognize this endemic clade as a single species complex, following the same criterion set for the North American fauna (Colbourne & Hebert, 1996). Similar to the *D. pulex* species complex in North America, this assemblage shows evidence of a recent radiation, which is accompanied by the same suite of traits that likely spurs diversification, yet has plagued taxonomists with uncertainties. Hybridization is commonly observed among five species (Hebert & Wilson, 1994). In one example (*D. thomsoni*), obligate parthenogenesis owes its origin to polyploidy via interspecific hybridization. In another instance (*Daphnia cephalata* King), obligate parthenogenesis has likely evolved *de novo*. Although asexuality is frequently observed in marginal and northern geographical areas (termed geographical parthenogenesis; Bell, 1982), and can have a significant phylogeographical component linked to particular geological events (Paland, Colbourne & Lynch, 2005), its independent origins in Australian and North American daphniids could implicate a more general set of conditions for its evolution.

Besides correctly assigning the narrowly endemic *D. jollyi* to the *D. carinata* complex, the present study provides clarification on the geographical origin and distribution of two transcontinental species, and reveals further flaws in the taxonomy of the subgenus. An earlier study of populations from four continents identified an abrupt genetic shift between Australian *D. lumholtzi* (Group 2) and isolates from Asia, Africa, and North America (Havel, Colbourne & Hebert, 2001). The application of a molecular clock dated this dispersal event at approximately 4 Mya, yet there was no evidence for the directionality of the colonization. Its consignment to a clade that includes *D. magna* from North America and *D. similis* from Israel suggests that *D. lumholtzi* does not originate from Australia but, instead, invaded its lakes long after the fragmentation of Gondwana. This finding, together with others (Weider *et al*., 1999; Adamowicz *et al*., 2002, 2004; Hebert, Witt & Adamowicz, 2003), confirms that dispersal events across large distances can result in the establishment of isolated populations that are free to independently evolve on separate continents. However, the unexpected phylogenetic positioning of *D. similis* (Israel) relative to the North American isolate clearly indicates a taxonomic error. Morphologically similar forms to the first described specimen by Claus (1876) from a pond near Jerusalem have been reported in Europe, Asia, and North America. Recent work on Eurasian (Hudec, 1991), North American (Hebert & Finston, 1993), and South American (Adamowicz *et al*., 2004) populations has indicated the presence of species complexes. Unfortunately, in the absence of genetic comparisons with specimens from the type locality, no assessment of common ancestry can be made. Hence, the present study shows that *D. similis* and *D. exilis* form a species complex including *Daphnia spinulata* (Birabén) (Adamowicz *et al*., 2004) that is endemic to North and South America and should be a focus of taxonomic reappraisal.

Finally, the Australian fauna includes a deeply divergent lineage containing two species that form the *D. citrina* species complex (Group 3). Two lines of evidence suggest that its origin predates the breakup of Gondwanaland and is thus unlikely to be endemic to this continent. First, this group shows genetic distances that are greater than those separating the endemic *D. carinata* species complex from ctenodaphniid lineages common in North America. The group's ancestry may stem from a branch of the phylogeny that roots species previously assigned to the genus *Daphniopsis*. Second, this group is allied with species belonging to the *Daphniopsis atkinsoni* (Baird) species complex, whose epicentre is the Mediterranean region. *Daphnia salina* from North America is a member of this group, and related species have been described in the saline waters of Argentina (Paggi, 1996). Although verification of these biogeographical patterns awaits the study of sequence diversity of *Daphnia* on other continents, the present phylogeny provides clues about the geological factors that have shaped the early diversification of the genus. Save for a single species representing the *Australodaphnia*, the Australian fauna is shown to be exclusively ctenodphniid. By contrast, this subgenus forms a minor element of the North American and European fauna. With the dominant subgenera on Laurasian landmasses absent from Australia, subgeneric boundaries were clearly established in the Mesozoic and linked to drifting tectonic plates. Despite evidence of significant divergence following dispersal events in the recent past (Taylor, Hebert & Colbourne, 1996; Colbourne

et al., 1998; Cerny & Hebert, 1999; Weider *et al*., 1999; Schwenk, Posada & Hebert, 2000; Adamowicz *et al*., 2002, 2004; Hebert, Witt & Adamowicz, 2003), daphniids have been remarkably ineffective in intercontinental movement.

In spite of their antiquity, there are few diagnostic morphological differences among the subgenera. The difficulty in identifying such traits is attributed, in part, to each subgenus (except *Australodaphnia*) consisting of a number of deeply divergent lineages, which themselves show considerable morphological diversity (Hebert, 1995). Convergent evolution is also an important complication with recurrent trait loss and acquisition in each subgenus (Colbourne *et al.*, 1997). For example, the *Daphnia* phylogeny now indicates that the absence of a female tail spine (which was a diagnostic feature of the paraphyletic species that belonged to *Daphniopsis*) was twice lost in lineages of *Ctenodaphnia*. Other traits, such as cuticular melanization, were independently gained in each of the four subgenera. Because of such complications, the examination of single morphological traits does not allow the unambiguous assignment of species to a particular subgenus. However, the joint inspection of three traits does appear to allow definitive assignments for all species of *Daphnia* (Table 6). The adequacy of this classification system can be tested by the extension of molecular analyses to daphniids from other continents.

The link between UV exposure and mutagenesis is well established; the potential responses by *Daphnia* (Gonçalves, Villafañe & Helbling, 2002) and effects in aquatic systems are under investigation (Häder & Sinha, 2005). UV stress is particularly intensified in saline habitats by their lack of humic acids and other photoprotective agents precipitated by salt (Fox, 1983). High salt concentrations can also devastate proteins and impair DNA replication. The results of the present study confirm earlier observations that halophilic *Daphnia* show dramatic increases in molecular evolutionary rates (Hebert *et al*., 2002). Although future molecular evolutionary studies to include halophilic ctenodaphniids from other continents (*Daphnia mediterranea* Alonso and *Daphnia menucoensis* Paggi) will broaden the comparative analysis, we are now able to determine the number of independent habitat shifts to saline and high UV environments from a combined analysis of Australian and North American species, to evaluate whether mutational stress arises from any one component. Diversification of these *Ctenodaphnia* involved three transitions into environments with ionic concentrations greater than 20 000 uS cm^{-1} and two transitions into UV-rich freshwaters. In each case, rates of divergence increased and adaptive responses likely evolved to combat the harmful effects of radiation (except *D. studeri*, which retained its ancestral habits). The most obvious phenotypic response is the deposition of melanin in the carapace, which varies among species from dark brown to coal black and serves an important role in protecting *Daphnia* from shortwave light (Hebert & Emery, 1990; Hessen *et al*., 1999).

It is tempting to conclude from this convergent pattern that *Daphnia* is predisposed to evolve a melanized cuticle when challenged by UV radiation. Although many other aquatic crustaceans sequester carotenoids for apparently the same purpose, these pigments seem to play a minor photoprotective role in *Daphnia* (Hessen, 2002). Besides, melanin production is common throughout the genus, within the eye and the epidermal tissue surrounding the ephippial (diapausing) egg chambers, and a study on the convergent evolution of sex-specific melanization in abdominal segments of a fellow arthropod (*Drosophila*) shows that genetic signalling pathways required for expressing associated genes in a tissue-specific manner can be conserved for long evolutionary periods (Gompel & Carroll, 2003). However, two saline lineages have accelerated molecular clocks and are surely exposed to UV stress; yet they do not possess a melanic carapace (Fig. 3). The present study also uncovers a freshwater species complex that cryptically exhibits a fast clock,

Table 6. Three traits that jointly permit the assignment of *Daphnia* species to a subgenus

Trait	Attribute	*Australodaphnia*	*Ctenodaphnia*	*Daphnia*	*Hyalodaphnia*
Male flagellum	Relative length of flagellum/aesthetasc	1	2–4	2	1
	Tip of flagellum	Spatulate	Linear, rarely spatulate	Linear, rarely spatulate	Linear
Ephippium	Number of egg chambers	1	1 or 2	2	2
	Position of egg chamber(s)	Horizontal	Angle	Vertical	Vertical
Female tail spine	Presence or absence	Present	Lost in species previously assigned to *Daphniopsis*	Present	Present

for there are no a priori indications that it is particularly susceptible to UV. Then again, the ephippia of the *D. citrina* complex are the only *Daphnia* propagules that lack melanin (they are orange in colour) and may therefore be prone to UV radiation. Research is underway (by J. K. C.) to reveal other evolutionarily conserved defense strategies used by these species to overcome this environmental contest, beginning with the characterization of candidate genes involved in photorepair of DNA and in eliminating damaging oxygen radicals induced by UV. If evolution is indeed paralleled in lineages occupying saline and UV-rich freshwaters, which has undoubtedly provided increased opportunities for divergence into unexploited habitats by *Daphniopsis*, the emerging transparent lakes on Antarctica are potential arenas for other daphniid adaptive mini-radiations. Interestingly, these novel habitats are currently occupied by *D. studeri*, which is the only cladoceran to have colonized these lakes (Pugh, Dartnall & McInnes, 2002), presumably because of stratagems that have enabled its survival in ancestral saline environments.

Concluding remarks

In summary, the present study is a comprehensive examination of the evolutionary history of *Daphnia* and *Daphniopsis* from Australia using DNA sequence information from three mitochondrial genes. Although the varying pace of molecular evolution and the deep genetic divergence of lineages impair our ability to identify a single tree-topology, the phylogenies resolve a longstanding taxonomic uncertainty concerning these two genera, by definitively assigning all species to the genus *Daphnia*. Taxa previously ascribed to the genus *Daphniopsis* form two groups within an enlarged subgenus *Ctenodaphnia*, which also includes a species earlier mistaken for a member of the subgenus *Daphnia*. In all, the Australian fauna is shown to contain five distinct lineages including an endemic species that is the sole representative of the subgenus *Australodaphnia*. Although continental isolation has clearly shaped the early diversification of the genus, the variety of distinct aquatic habitats found in Australia has promoted speciation in the *Ctenodaphnia* similar to the mini-radiations seen for *Daphnia* on other continents. This is particularly true within saline waters, which are important components of this arid continent. The molecular data provide additional evidence of habitat-specific rates of evolution, extending observations of an accelerated molecular clock to all lineages exposed to intense UV radiation in both saline and transparent oligohaline-freshwater environments. Convergent adaptive traits associated with living in these harsh milieux seem apparent. Yet, knowledge on the conservation of genetic mechanisms acting to promote such dramatic shifts is needed to advance our understanding of why *Daphnia* are such proficient exploiters of the full spectrum of inland aquatic habitats.

ACKNOWLEDGEMENTS

This research was supported by a NSERC research grant to P. Hebert, a NSF grant to M. Lynch, and by postdoctoral fellowships to C. Wilson at the Australian National University (NSERC and ARC awards) and to J. Colbourne at the University of Oregon (NSERC award). Computer support was provided by the Center for Genomics and Bioinformatics and the Indiana University Information Technology Services, which are supported in part by the Indiana Genomics Initiative (INGEN) under the Lilly Endowment. We thank the Research School of Biological Sciences at the Australian National University for access to research facilities and field support. B. Williams and S. S. Schwartz kindly provided specimens of *D. tibetana*, *D. similis* from Israel and *D. studeri*. Thanks to E. A. Remigio for sequencing key specimens of the *D. carinata* complex and to S. J. Adamowicz, G. Stuart, and two anonymous reviewers for their helpful comments on earlier drafts of this manuscript.

NOTE ADDED IN PROOF

The authors apologize to colleagues whose recent contributions were not cited due to delays in publishing this manuscript.

REFERENCES

Adamowicz SJ, Gregory TR, Marinone MC, Hebert PDN. 2002. New insights into the distribution of polyploid *Daphnia*: the Holarctic revisited and Argentina explored. *Molecular Ecology* **11:** 1209–1217.

Adamowicz SJ, Hebert PDN, Marinone MC. 2004. Species diversity and endemism in the *Daphnia* of Argentina: a genetic investigation. *Zoological Journal of the Linnean Society* **140:** 171–205.

Akaike H. 1974. A new look at the statistical model identification. *IEEE Transactions on Automatic Control* **19:** 716–723.

Arts MT, Robarts RD, Kasai F, Waiser MJ, Tumber VP, Plante AJ, Rai H, de Lange HJ. 2000. The attenuation of ultraviolet radiation in high dissolved organic carbon waters of wetlands and lakes on the northern Great Plains. *Limnology and Oceanography* **45:** 292–299.

Bell G. 1982. *The masterpiece of nature*. San Francisco, CA: University of California Press.

Benzie JAH. 1986. *Daphnia occidentalis*, new species (Cladocera: Daphniidae) from western Australia: new evidence on the evolution of the North American *Daphnia ambigua* – *Daphnia middendorffiana* group. *Journal of Crustacean Biology* **6:** 232–245.

Benzie JAH. 1988a. The systematics of Australian *Daphnia* (Cladocera: Daphnidae). Multivariate morphometrics. *Hydrobiologia* **166:** 163–182.

Benzie JAH. 1988b. The systematics of Australian *Daphnia* (Cladocera: Daphnidae). Species descriptions and keys. *Hydrobiologia* **166:** 95–161.

Benzie JAH. 1988c. The systematics of Australian *Daphnia* (Cladocera: Daphnidae). Electrophoretic analyses of the *Daphnia carinata* complex. *Hydrobiologia* **166:** 183–197.

Benzie JAH, Bayly IAE. 1996. Male and ephippial female of *Daphnia jollyi* Petkovski, 1973 discovered in Western Australia and the parthenogenetic female redescribed. *Hydrobiologia* **331:** 171–181.

Benzie JAH, Hodges AMA. 1996. *Daphnia obtusa* Kurz, 1874 Emend Scourfield, 1942 from Australia. *Hydrobiologia* **333:** 195–199.

Bremer K. 1994. Branch support and tree stability. *Cladistics* **10:** 295–304.

Broughton RE, Cott S, Stanley E, Durrett RT. 2000. Quantification of homoplasy for nucleotide transitions and transversions and a reexamination of assumptions in weighted phylogenetic analysis. *Systematic Biology* **49:** 617–627.

Buckley TR, Simon C, Chambers GK. 2001. Exploring among-site rate variation models in a maximum likelihood framework using empirical data: effects of model assumptions on estimates of topology, branch lengths, and bootstrap support. *Systematic Biology* **50:** 67–86.

Cerny M, Hebert PDN. 1999. Intercontinental allozyme differentiation among four holarctic *Daphnia* species. *Limnology and Oceanography* **44:** 1381–1387.

Claus C. 1876. Zur Kenntniss der Organization und des feinern Baues des Daphniden und verwandter Cladoceren. *Zeitschrift für Wissenschaftliche Zoologie* **27:** 362–402.

Colbourne JK, Crease TJ, Weider LJ, Hebert PDN, Dufresne F, Hobæk A. 1998. Phylogenetics and evolution of a circumarctic species complex (Cladocera: *Daphnia pulex*). *Biological Journal of the Linnean Society* **65:** 347–365.

Colbourne JK, Hebert PDN. 1996. The systematics of North American *Daphnia* (Crustacea: Anomopoda): a molecular phylogenetic approach. *Philosophical Transactions of the Royal Society of London Series B* **351:** 349–360.

Colbourne JK, Hebert PDN, Taylor DJ. 1997. Evolutionary origins of phenotypic diversity in *Daphnia*. In: Givnish, TJ, Sytsma, KJ, eds. *Molecular evolution and adaptive radiation*. Cambridge: Cambridge University Press, 163–188.

Conant G, Lewis PO. 2001. Effects of nucleotide composition bias on the success of the parsimony criterion in phylogenetic inference. *Molecular Biology and Evolution* **18:** 1024–1033.

Crease TJ. 1999. The complete sequence of the mitochondrial genome of *Daphnia pulex* (Cladocera: Crustacea). *Gene* **233:** 89–99.

De Rijk P, De Wachter R. 1993. DCSE, an Interactive tool for sequence alignment and secondary structure research. *CABIOS* **9:** 735–740.

Eriksson T. 1995. *AutoDecay v.2.9.8.* Available at http://www.botan.su.se/systematik/folk/Torsten.html.

Fox LE. 1983. The removal of dissolved humic acid during estuarine mixing. *Estuarine Coastal Shelf Science* **16:** 431–440.

Fryer G. 1991a. Functional morphology and the adaptive radiation of the Daphniidae (Branchiopoda: Anomopoda). *Philosophical Transactions of the Royal Society of London Series B* **331:** 1–99.

Fryer G. 1991b. A daphniid ephippium (Branchiopoda: Anomopoda) of Cretaceous age. *Zoological Journal of the Linnean Society* **102:** 163–167.

Galtier N, Gouy M. 1995. Inferring phylogenies from DNA sequences of unequal base compositions. *Proceedings of the National Academy of Sciences of the United States of America* **92:** 11317–11321.

Giessler S, Mader E, Schwenk K. 1999. Morphological evolution and genetic differentiation in *Daphnia* species complexes. *Journal of Evolutinary Biology* **12:** 710–723.

Gompel N, Carroll SB. 2003. Genetic mechanisms and constraints governing the evolution of correlated traits in drosophilid flies. *Nature* **424:** 931–935.

Gonçalves RJ, Villafañe VE, Helbling EW. 2002. Photorepair activity and protective compounds in two freshwater zooplankton species (*Daphnia menucoensis* and *Metacyclops mendocinus*) from Patagonia, Argentina. *Photochemical and Photobiological Sciences* **1:** 996–1000.

Häder D-P, Sinha RP. 2005. Solar ultraviolet radiation-induced DNA damage in aquatic organisms: potential environmental impact. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis* **571:** 221–233.

Hann BJ. 1986. Revision of the genus *Daphniopsis* (Sars, 1903) (Cladocera: Daphniidae) and a description of *Daphniopsis chilensis*, new species, from South America. *Journal of Crustacean Biology* **6:** 246–263.

Havel JE, Colbourne JK, Hebert PDN. 2001. Reconstructing the history of intercontinental dispersal in *Daphnia lumholtzi* by use of genetic markers. *Limnology and Oceanography* **45:** 1414–1419.

Hebert PDN. 1977. A revision of the taxonomy of the Genus *Daphnia* (Crustacea: Daphnidae) in south-eastern Australia. *Australian Journal of Zoology* **25:** 371–398.

Hebert PDN. 1995. *The Daphnia of North America: an illustrated fauna* [CD-ROM distributed by the author]. Guelph: Department of Zoology, University of Guelph.

Hebert PDN, Emery CJ. 1990. The adaptive significance of cuticular melanization in *Daphnia*. *Functional Ecology* **4:** 703–710.

Hebert PDN, Finston TL. 1993. A taxonomic reevaluation of North American *Daphnia* (Crustacea: Cladocera). I. The *D. similis* complex. *Canadian Journal of Zoology* **71:** 908–925.

Hebert PDN, Remigio EA, Colbourne JK, Taylor DJ, Wilson CC. 2002. Accelerated molecular evolution in halophilic crustaceans. *Evolution* **556:** 909–926.

Hebert PDN, Wilson CC. 1994. Provincialism in plankton: endemism and allopatric speciation in *Daphnia*. *Evolution* **48:** 1333–1349.

Hebert PDN, Wilson CC. 2000. Diversity of the genus *Daphniopsis* in the saline waters of Australia. *Canadian Journal of Zoology* **78:** 794–808.

Hebert PDN, Witt JDS, Adamowicz SJ. 2003. Phylogeographical patterning in *Daphnia ambigua*: regional

divergence and intercontinental cohesion. *Limnology and Oceanography* **48:** 261–268.

Hessen DO. 2002. UV radiation and arctic freshwater zooplankton. In: Hessen, DO, ed. *UV radiation and arctic ecosystems: ecological studies*, Vol. 153. Heidelberg: Springer-Verlag, 158–184.

Hessen DO, Borgeraas J, Kessler K, Refseth UH. 1999. UV-B susceptibility and photoprotection of Arctic *Daphnia* morphotypes. *Polar Research* **18:** 345–352.

Hessen DO, Rukke NA. 2000. UV radiation and low calcium as mutual stressors for *Daphnia*. *Limnology and Oceanography* **45:** 1834–1838.

Hillis DM, Huelsenbeck JP. 1992. Signal, noise, and reliability in molecular phylogenetic analyses. *Journal of Heredity* **83:** 189–195.

Hrbácek J. 1987. Systematics and biogeography of *Daphnia* species in the northern temperate region. *Memorie Dell'istituto Italiano Di Idrobiologia* **45:** 37–76.

Hudec I. 1991. A comparison of populations from the *Daphnia similis* group (Cladocera: Daphniidae). *Hydrobiologia* **225:** 9–22.

Huelsenbeck JP, Larget B, Miller RE, Ronquist F. 2002. Potential applications and pitfalls of Bayesian inference of phylogeny. *Systematic Biology* **51:** 673–688.

Huelsenbeck JP, Ronquist F. 2001. MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* **17:** 754–755.

Kimura M. 1980. A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution* **16:** 111–120.

Korovchinsky NM. 1997. On the history of studies on cladoceran taxonomy and morphology, with emphasis on early work and causes of insufficient knowledge of the diversity of the group. *Hydrobiologia* **360:** 1–11.

Kumar S, Tamura K, Jakobsen IB, Nei M. 2001. MEGA2: molecular evolutionary genetics analysis software. *Bioinformatics* **17:** 1244–1245.

Li P, Bousquet J. 1992. Relative-rate test for nucleotide substitutions between lineages. *Molecular Biology and Evolution* **9:** 1185–1189.

Omilian AR, Taylor DJ. 2001. Rate acceleration and long-branch attraction in a conserved gene of cryptic daphniid (Crustacea) species. *Molecular Biology and Evolution* **18:** 2201–2212.

Paggi JC. 1996. *Daphnia* (*Ctenodaphnia*) *menucoensis* (Anomopoda Daphniidae) – a new species from athalassic saline waters in Argentina. *Hydrobiologia* **319:** 137–147.

Paland S, Colbourne JK, Lynch M. 2005. Evolutionary history of contagious asexuality in *Daphnia pulex*. *Evolution* **59:** 800–813.

Philippe H, Lecointre G, Le HLV, Le Guyader H. 1996. A critical study of homoplasy in molecular data with the use of a morphologically based cladogram, and its consequences for character weighting. *Molecular Biology and Evolution* **13:** 1174–1186.

Posada D, Crandall KA. 1998. MODELTEST: testing the model of DNA substitution. *Bioinformatics* **14:** 817–818.

Pugh PJA, Dartnall HJG, McInnes SJ. 2002. The non-marine Crustacea of Antarctica and the Islands of the Southern Ocean: biodiversity and biogeography. *Journal of Natural History* **36:** 1047–1103.

Robinson M, Gouy M, Gautier C, Mouchiroud D. 1998. Sensitivity of the relative-rate test to taxonomic sampling. *Molecular Biology and Evolution* **15:** 1091–1098.

Robinson-Rechavi M, Huchon D. 2000. RRTree: Relative-rate tests between groups of sequences on a phylogenetic tree. *Bioinformatics* **16:** 296–297.

Rühe FE. 1914. Die Süsswassercrustaceen die Deutschen Sudpolarexpedition 1901–03 mit Ausschluss der Ostracoden. *Deutsche Sudpolar-Expedition 16 Zoology* **8:** 5–66.

Sars GO. 1903. On the crustacean fauna of central Asia. Part 2. Cladocera. *Annuaire Du Musée Zoologique St Petersburg (Akademiia Nauk)* **8:** 157–194.

Sars GO. 1914. *Daphnia carinata* King and its remarkable varieties. *Archiv für Mathematik Og Naturvidenskab B* **34:** 1–14.

Schwartz SS, Hebert PDN. 1984. *Daphniopsis ephemeralis* sp. n. (Cladocera, Daphniidae): a new genus for North America. *Canadian Journal of Zoology* **63:** 2689–2693.

Schwenk K, Posada D, Hebert PDN. 2000. Molecular systematics of European *Hyalodaphnia*: the role of contenporary hybridization in ancient species. *Proceedings of the Royal Society of London Series B* **267:** 1833–1842.

Siddall ME. 1995a. Another monophyly index. Revisiting the jackknife. *Cladistics* **11:** 33–56.

Siddall ME. 1995b. *Random cladistics*, Version 3.0. Available by ftp at zoo.toronto.edu.

Smirnov NN. 1992. Mesozoic Anomopoda (Crustacea) from Mongolia. *Zoological Journal of the Linnean Society* **104:** 97–116.

Strimmer K, von Haeseler A. 1997. Likelihood-mapping: a simple method to visualize phylogenetic content of a sequence alignment. *Proceedings of the National Academy of Sciences of the United States of America* **94:** 6815–6819.

Swain TD, Taylor DJ. 2003. Structural rRNA characters support monophyly of raptorial limbs and paraphyly of limb specialization in water fleas. *Proceedings of the Royal Society of London Series B* **270:** 887–896.

Swofford DL. 2003. *PAUP* phylogenetic analysis using parsimony (*and other methods)*, Version 4. Sunderland, MA: Sinauer Associates.

Swofford DL, Waddell PJ, Huelsenbeck JP, Foster PG, Lewis PO, Rogers JS. 2001. Bias in phylogenetic estimation and its relevance to the choice between parsimony and likelihood methods. *Systematic Biology* **50:** 525–539.

Tarrío R, Rodríguez-Trelles F, Ayala FJ. 2001. Shared nucleotide composition biases among species and their impact on phylogenetic reconstructions of the Drosophilidae. *Molecular Biology and Evolution* **18:** 1464–1473.

Taylor DJ, Crease TJ, Brown WM. 1999. Phylogenetic evidence for a single long-lived clade of crustacean cyclic parthenogens and its implications far the evolution of sex. *Proceedings of the Royal Society of London Series B* **266:** 791–797.

Taylor DJ, Finston TL, Hebert PDN. 1998. Biogeography of a widespread freshwater crustacean: pseudocongruence and cryptic endemism in the North American *Daphnia laevis* complex. *Evolution* **52:** 1648–1670.

Taylor DJ, Hebert PDN, Colbourne JK. 1996. Phylogenetics and evolution of the *Daphnia longispina* group (Crustacea) based on 12S rDNA sequence and allozyme variation. *Molecular Phylogenetics and Evolution* **5:** 495–510.

Thompson JD, Higgins DG, Gibson TJ. 1994. ClustalW: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position specific gap penalties and weight matrix choice. *Nucleic Acids Research* **22:** 4673–4680.

Van de Peer Y, Robbrecht E, de Hoog S, Caers A, De Rijk P, De Wachter R. 1999. Database on the structure of small subunit ribosomal RNA. *Nucleic Acids Research* **27:** 179–183.

Wagler E. 1936. Die Systematik und geographische Verbreitung des Genus *Daphnia* O.F. Müller mit besonderer Berücksichtigung der südafrikanishen *Arten*. *Archiv für Hydrobiologie* **30:** 505–556.

Weider LJ, Hobaek A, Colbourne JK, Crease TJ, Dufresne F, Hebert PDN. 1999. Holarctic phylogeography of an asexual species complex I. Mitochondrial DNA variation in arctic *Daphnia*. *Evolution* **53:** 777–792.

Yang Z. 1996. Among-site rate variation and its impact on phylogenetic analyses. *Trends in Ecology and Evolution* **11:** 367–372.

Yang Z. 1998. On the best evolutionary rate for phylogenetic analysis. *Systematic Biology* **47:** 125–133.

Yang Z, Goldman N, Friday A. 1994. Comparison of models for nucleotide substitution used in maximum-likelihood phylogenetic estimation. *Molecular Biology and Evolution* **11:** 316–324.

Zhang JZ. 1999. Performance of likelihood ratio tests of evolutionary hypotheses under inadequate substitution models. *Molecular Biology and Evolution* **16:** 868–875.