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Molecular phylogeny and biogeography of spring-associated hydrobiid snails of the Great Artesian Basin, Australia

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Abstract

The Great Artesian Basin (GAB) of Australia underlies some of the driest parts of South Australia and Queensland and feeds numerous freshwater springs. Prominent and endangered components of the GAB spring community are snails of the family Hydrobiidae. This paper examines the evolutionary relationships of the entire hydrobiid fauna associated with the GAB, and includes appropriate non-GAB species to place the GAB fauna in a broader phylogenetic context. The Queensland genus *Jardinella* is a focus of this paper, providing a fine scale examination of relationships between spring supergroups in the northeastern regions of the GAB. Maximum parsimony and Bayesian analyses performed on 16S, CO1, and combined sequence data from 40 hydrobiid taxa found four major clades of Australian taxa. The analysis revealed that at least three separate colonization events of the GAB spring fauna have occurred. Two of these are represented by considerable radiations, (1) *Jardinella* to the north and east and (2) *Caldicochlea*, *Fonscochlea*, and possibly *Trochidrobia* in South Australia. The phylogenetic position of the latter is uncertain so it may represent yet another invasion. The third definite invasion is represented by a single species of the speciose SE Australian genus *Austropyrgus* in the Dalhousie Springs in South Australia. *Jardinella* is found to be monophyletic, and with one exception, its members in each of the Queensland spring supergroups are found to be monophyletic.

Keywords: Hydrobiidae; Australia; Great Artesian Basin; mtDNA; 16S; CO1; Phylogeny

1. Introduction

The Great Artesian Basin (GAB) of Australia is the largest artesian system in the world. Numerous freshwater springs are found associated with the GAB in South Australia and Queensland. These springs persist in some of the driest parts of Australia. Because these springs are fed by continuous seepage from the artesian basin the water supply is continuous, providing a unique set of small oasis-like environments in an area with few other permanent bodies of water. The springs are of considerable conservation interest due to the presence of endemic plants and animals including fishes, crustaceans, and flatworms (Ponder, 1986, 2004), with the majority of known endemics being snails of the family Hydrobiidae (Ponder, 1995, 2004; Ponder and Clark, 1990; Ponder et al., 1989, 1995). Hydrobiids are the most diverse of all freshwater gastropods, with nearly 400 valid generic names currently in use (Kabat and Hershler, 1993). Because of their extremely limited dispersal capabilities,

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they often become isolated resulting in small-scale allopatric speciation (e.g., Clark et al., 2003; Ponder, 1982; Ponder and Colgan, 2002; Ponder et al., 1993) making them excellent candidates for evolutionary, biogeographic, and ecological studies (Hurt, 2004; Ponder and Colgan, 2002; Ponder et al., 1994, 1996). Wilke et al. (2001) have shown that the Hydrobiidae is polyphyletic and we are using the name in the general sense. The formal higher-level taxonomy of hydrobiids worldwide is currently in flux and is not an issue this paper can address.

Research over the previous decade conducted largely by Ponder, Colgan, and colleagues (e.g., Ponder et al., 1989, 1994, 1995, 1996) has shown that the springs maintain a large fauna of approximately 23+ species and five genera, three of which are endemic to the GAB. Detailed anatomical studies revealed that most springs appeared to support several species typically restricted to a single spring or spring group. It has been hypothesized that as the region became more arid (DeDeckker, 1986; Kemp, 1978; Ponder, 1986), populations of poorly dispersing aquatic animals requiring long-term permanent water became isolated from one another as non-artesian water bodies dried. The relicts became isolated in the refuges provided by the permanent water in the often widely separated artesian springs or clusters of springs and eventually evolved into distinct taxa.

Springs in the GAB cluster into aggregations termed supergroups (SG) (Ponder, 1986). These SG's are separated by distances of 200 km or more and each has a characteristic complement of snails. The Dalhousie Springs SG of northern South Australia has two genera, *Austropyrgus* with one species and the endemic *Caldicochlea* with two species (Colgan and Ponder, 2000; Ponder et al., 1996). The Lake Eyre SG (Ponder et al., 1989, 1995) has two endemic genera, *Trochidrobia* and *Fonscochlea*. A single morphologically diverse genus, *Jardinella*, with 12 described species is recognized from four of the Queensland spring SGs. Five or six recognized SGs (Fensham and Fairfax, 2003; Ponder, 1986) are not known to contain hydrobiids (Ponder and Clark, 1990).

The systematic and genetic studies to date (see above) have resulted in a better understanding of the biological richness and patterns of speciation of hydrobiids within the two major groups of artesian springs associated with GAB in South Australia—the Lake Eyre Supergroup (Habermehl, 1982; Smith, 1989) and Dalhousie Springs, of northern South Australia (Zeidler and Ponder, 1989). In contrast, the hydrobiid faunas of the springs in western Queensland are known from only one published study (Ponder and Clark, 1990), a morphological assessment of material from two collecting trips in the 1980s. Recent surveys in Queensland springs have found additional Queensland spring taxa that have yet to be formally named, many are used in this paper to examine relationships in the genus *Jardinella*, however, no taxonomic names are established herein. No previous studies of the genetics of the Queensland GAB associated snails have been conducted.

This paper is addressed at understanding the evolutionary relationships of the GAB hydrobiids. These relationships currently are largely unknown. For instance, it is not known whether the congeneric taxa in each separate spring SG radiated from a single shared common ancestor, or whether they are derived from multiple ancestors. With the probable exception of a species of the SE Australian genus *Austropyrgus* in Dalhousie Springs in South Australia (Ponder et al., 1996), it is unclear whether or not the rest of the GAB hydrobiid fauna represents more than one isolation or dispersal event from non-artesian freshwater habitats. Such questions will be addressed by examining the GAB fauna and appropriate non-GAB hydrobiid species in a broad phylogenetic context.

2. Materials and methods

2.1. Specimens and vouchers

GAB hydrobiids examined in this study were collected primarily during January–May 2001, and some South Australian specimens were collected in the early 1990s. Table 1 lists the taxa analyzed, collection sites, and the Australian Museum-Sydney (AMS) accession numbers. Individual sequences and the sequence alignment are available on GenBank, CO1: AY622436–AY622486, 16S: AY622382–AY622435. Fig. 1 indicates sampling localities of each species to provide some representative scale for comparing GAB to non-GAB taxa and indicate the spring supergroups from which *Jardinella* were collected. Sequence for one ingroup taxon was obtained from GenBank, *Phrantela marginata* CO1: AF129331. Sequences from the outgroup species, *Bithynia tentaculata* CO1: AF445334, 16S: AF445344; *Bythinella pannonica* CO1: AY222650, 16S: AY222660; *Cincinnatia winkleyi* CO1: AF118370, 16S: AF118370; *Gammatricula fujianensis* CO1: AF213342, 16S: AF212896; *Horatia sturmi* CO1: AF213345, 16S: AF212899; *Hydrobia glyca* CO1: AF247798; 16S: AF478397, *Mercuria emiliana* CO1: AF213346, 16S: AF212900; and *Amnicola limosa* CO1: AF213348, 16S: AF212903 were obtained from GenBank.

2.2. DNA extraction, PCR amplification, and sequencing

The samples used in this study were preserved in 70% ethanol or kept frozen. Non-*Jardinella* samples were treated as in Colgan et al. (2003) and *Jardinella* specimens were treated as follows. Total genomic DNA was extracted from several milligrams of tissue by

Table 1
List of taxa analyzed

Taxa	Location	Australian Museum Accession No.
Great Artesian Basin Taxa		
Austropyrgus centralia	Dalhousie Springs, spring Cd11, SA	C.201748
Caldicochlea globosa	Dalhousie Springs, Spring CA1, SA	C.435869, C.435870
Caldicochlea harrisi	Dalhousie Springs, Spring CA1, SA	C.435870, C.435871
Fonscochlea variabilis	Station LA 13B, SA	C.435868
Fonscochlea zeidleri	Strangways Spring, fenced, SA	C.435867
Jardinella acuminata	Edgbaston Springs, Big Spring, ~3 km SE of Edgbaston HS, QMS-11B, QLD	C.400139
Jardinella coreena	Edgbaston Springs, "Coreena" HS, large spring behind HS, QMS-17B, QLD	C.400146
Jardinella corrugata	"Group 1" springs, NE Edgbaston Springs, QMS-14, QLD	C.400143
Jardinella corrugata	Edgbaston Springs, Gum Hollow Spring, QMS-17A, QLD	C.400145
Jardinella edgbastonensis	Edgbaston Springs, Big Spring, ~3 km SE of Edgbaston HS, QMS-11C, QLD	C.400137
Jardinella isolata	Elizabeth Springs, 26 km NNW of Springvale, QLD	C.305895
Jardinella jesswiseae	Edgbaston Springs, Big Spring, about 3 km SE of Edgbaston HS, QMS-11B, QLD	C.400138
Jardinella pallida	Edgbaston Springs, Big Spring, about 3 km SE of Edgbaston HS, QMS-11B, QLD	C.400140
Jardinella tumorosa	Little Mulgrave River at base of escarpment, QLD	C.424635
Jardinella carnarvonensis	Carnarvon Gorge NP, Violet Gorge, "moss garden" QMS-20, QLD	C.400125
Jardinella nsp. QMS-21	Carnarvon NP, Carnarvon Gorge, small creek on sides of path emerging from N side of gorge, QMS-21, QLD	C.400126
Jardinella nsp. umb QMS-06	Bundoona Springs, QMS-06, QLD	C.400127
Jardinella nsp. QMS-06	Bundoona Springs, QMS-06, QLD	C.400128
Jardinella nsp. QMS-06A	Bundoona Springs, approx 50 m from station 6, QMS-06A, QLD	C.400129
Jardinella nsp. QMS-05	Bundoona Springs, QMS-05, QLD	C.400130
Jardinella nsp.uncoil QMS-04	Bundoona Springs, QMS-04, QLD	C.400131
Jardinella nsp. keel QMS-04	Bundoona Springs, QMS-04, QLD	C.400132
Jardinella nsp. umb QMS-04	Bundoona Springs, QMS-04, QLD	C.400133
Jardinella eulo	Spring near Tunga Bore, QMS-07, QLD	C.400135
Trochidrobia punicea	Finniss Springs West, SA	C.435866
Non-GAB taxa		
Austropyrgus angasi	Campaspe R, 19 km N Kyneton, VIC	C.166840
Austropyrgus cooma	Trib of Cooma Ck, 13.7 km S of Cooma on Myalla Rd, NSW	C.173969
Austropyrgus grampianensis	Dairy Creek, Grampians, VIC	C.302381
Austropyrgus rectus	Squeaky Beach Ck c. 60 m from rd upstream on N side (approx 37A), Wilsons Promontory NP, VIC	C.174026
Austropyrgus simsonianus	Jordan R, at Brighton, TAS	C.204041
Austropyrgus sparsus	Trib of Shoalhaven R, 41 km S. of Braidwood, NSW	C.173981
Austropyrgus turbatus	Squeaky Beach Ck c. 60 m from rd upstream on N side, Wilsons Promontory NP, VIC	C.174025
Beddomeia launcestonensis	South Esk R, ca 1.2 km below Trevallyn Dam, TAS	C.165671
Beddomeia hullii	Thirteen Mile Ck, TAS	C.203773
Beddomeia cf minima	St Patricks R, Tasman Hwy, TAS	C.165642
Nanocochlea nsp.	Damper Creek, Precipitious Bluff, TAS	C.203673
Phrantela daveyensis tristis	Trib. of Dismal Ck, trib. of Hardwood R, TAS	C.165893
Phrantela marginata	Thirteen Mile Ck, TAS	C.165881
Posticobia brazieri	Clarence River, Grafton, NSW	C.203018
Pseudotricula nsp.	Bauhaus Cave, Precipitious Bluff, TAS	C.201822
Westropyrgus westralis	Ellenbrook, WA	C.424647

Australian state abbreviations: Victoria, VIC; New South Wales, NSW; Tasmania, TAS; South Australia, SA; Queensland, QLD; West Australia, WA; South Australia, SA.

digestion of the entire animal with CTAB lysis buffer (Saghai-Maroof et al., 1984) and 10 μL Proteinase K (10 μg/mL) and then purified by phenol–chloroform extraction according to standard procedures (Hillis and Mortiz, 1990). DNA was precipitated using isopropanol and resuspended in 30 μL Tris–EDTA with RNase.

For amplification of partial mitochondrial 16S rDNA we used the16sar and 16sbr primers (Hillis and Mortiz, 1990); CO1H2198 and CO1L1490 were used to amplify partial cytochrome *c* oxidase-1 (CO1) sequences (Folmer et al., 1994). Approximately 10 ng of genomic DNA provided templates for double-stranded reactions via the polymerase chain reaction (PCR). PCRs were performed in a 25 μL solution containing each dNTP at 0.22 μM, each primer at 0.1 μM, 1.5 mM $MgCl_2$, 1 U *Taq* DNA polymerase, and 1× PCR buffer (without added $MgCl_2$). Reactions were amplified for 5 cycles of 92 °C for 40 s, 40 °C for 40 s, and 72 °C for 90 s followed by 30 cycles of 92 °C for 40 s, 50 °C for 40 s, and 72 °C for 90 s. Samples were purified and double-stranded DNA provided the template for cycle-sequencing using BigDye 3.1 (ABI) chemistry. Both strands were sequenced, using the initial

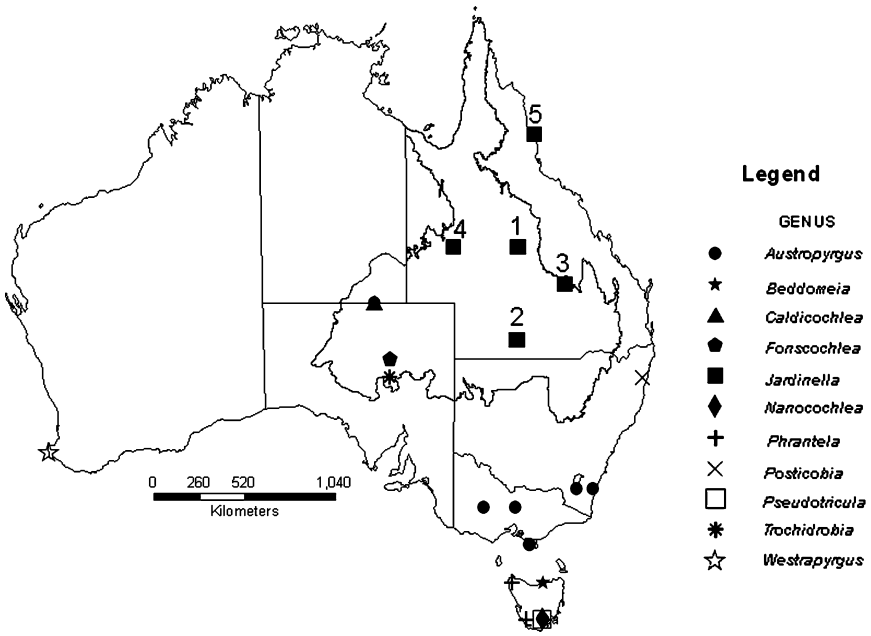

Fig. 1. Map of the Australian hydrobiid fauna examined in this study. The Great Artesian Basin is shown to highlight the identity of GAB and non-GAB fauna. The GAB is bounded on the east by the Great Dividing Range. Genera are indicated by a variety of symbols (on legend). *Jardinella* groups are numbered as follows: 1, Barcaldine SG; 2, Eulo SG; 3, Springsure SG; 4, Springvale SG; and 5, Little Mulgrave River.

amplification primers, to control sequence accuracy and to resolve any ambiguous bases. The products of cycle sequencing were cleaned using DyeEx spin cartridges and then dried in a rotary evaporator at 60 °C under vacuum. Samples were rehydrated in formamide and sequencing was performed on an ABI3100 automated sequencer according to manufacturer's directions.

2.3. Data analysis and phylogenetic reconstruction

Sequences of both strands were compared to each other and contigs were assembled in Sequencher 4.0.5 (Gene Codes, Ann Arbor, MI). Sequences were aligned by eye in BioEdit (Hall, 1999) with reference to secondary structure models to refine the alignment and identify regions corresponding to loops and stems (Lydeard et al., 2000). Aligned CO1, 16S, and combined sequences were analyzed using maximum parsimony with PAUP*4.0b10 (Swofford, 2002) using a heuristic search (100 addition replicates). The following options were used: uninformative characters were ignored; only minimal trees were kept, gaps were examined both as missing and as a 5th base (in separate analyses), and zero length branches were collapsed. For the combined analysis taxa were only used if sequences for both genes were available. Phylogenetic trees were rooted using the outgroup individuals mentioned previously. Support values for internal nodes of the trees were estimated using a bootstrap analysis with 10,000 pseudoreplicates (Felsenstein, 1985). Bremer support values (Bremer, 1994) were calculated using TreeRot.v2 (Sorenson, 1999). Shimodaira–Hasegawa (SH) tests (Shimodaira and Hasegawa, 1999), as implemented in PAUP* were used to test the monophyly of several clades, including the GAB fauna and the genus *Jardinella*, by comparing the likelihoods of constrained to unconstrained topologies. Constraint trees were constructed in MacClade 4.03 (Maddison and Maddison, 2000). Heuristic MP searches with 100 random-taxon-addition replicates were performed in PAUP* to obtain the shortest constrained and unconstrained topologies. ML parameters from analyses of the combined sequences in Modeltest 3.06 (Posada and Crandall, 1998) were used in the SH test in the context of the MP topologies. An additional analysis was performed on a limited combined dataset restricted to the spring taxa, excluding taxa that could be affected by saturation effects.

For Bayesian analysis, the 16S and CO1 data matrices were analyzed separately and together using Modeltest 3.06 to determine an appropriate model of evolution (GTR + I + G) for both genes followed by MCMCMC Bayesian analysis using MrBayes 3.0 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003) to estimate the posterior probability distribution. For the combined analysis, data were partitioned into 16S, CO1:1st, 2nd, and 3rd codon position to allow changes for each to be examined separately. For each analysis four Bayesian analyses with 500,000 generations were run each with a 10,000 generation burnin and four separate, differentially heated chains. For determination of the burnin (the number of generations before approximate stationarity) a plot of overall model likelihood against generation of the chain was examined to find the point where the likelihood levelled off and began to

fluctuate around a stable value. Final results were based on the pooled samples from the stationary phase of the run and trees from the burnin were excluded from the consensus trees.

3. Results

3.1. Sequence data

The regions of 16S rDNA and CO1 that were sequenced resulted in a combined aligned data matrix of 1027 bp (493 bp 16S rDNA; 534 bp CO1). Average base frequencies for CO1 were 0.25% A, 0.39% T, 0.17% C, and 0.20% G, for 16S frequencies were 0.39% A, 0.31% T, 0.14% C, and 0.19% G. An analysis of the 16S data are shown in Fig. 2A (56 taxa, 496 characters, and 169 parsimony-informative) using maximum parsimony resulted in 2399 trees (688 steps, CI = 0.4174—including uninformative characters). An analysis of the CO1 data are shown in Fig. 2B (49 taxa, 534 characters, and 214 parsimony-informative) using maximum parsimony resulted in 18 trees (1501 steps, CI = 0.2938). The combined analysis (37 taxa, 1027 characters, and 364

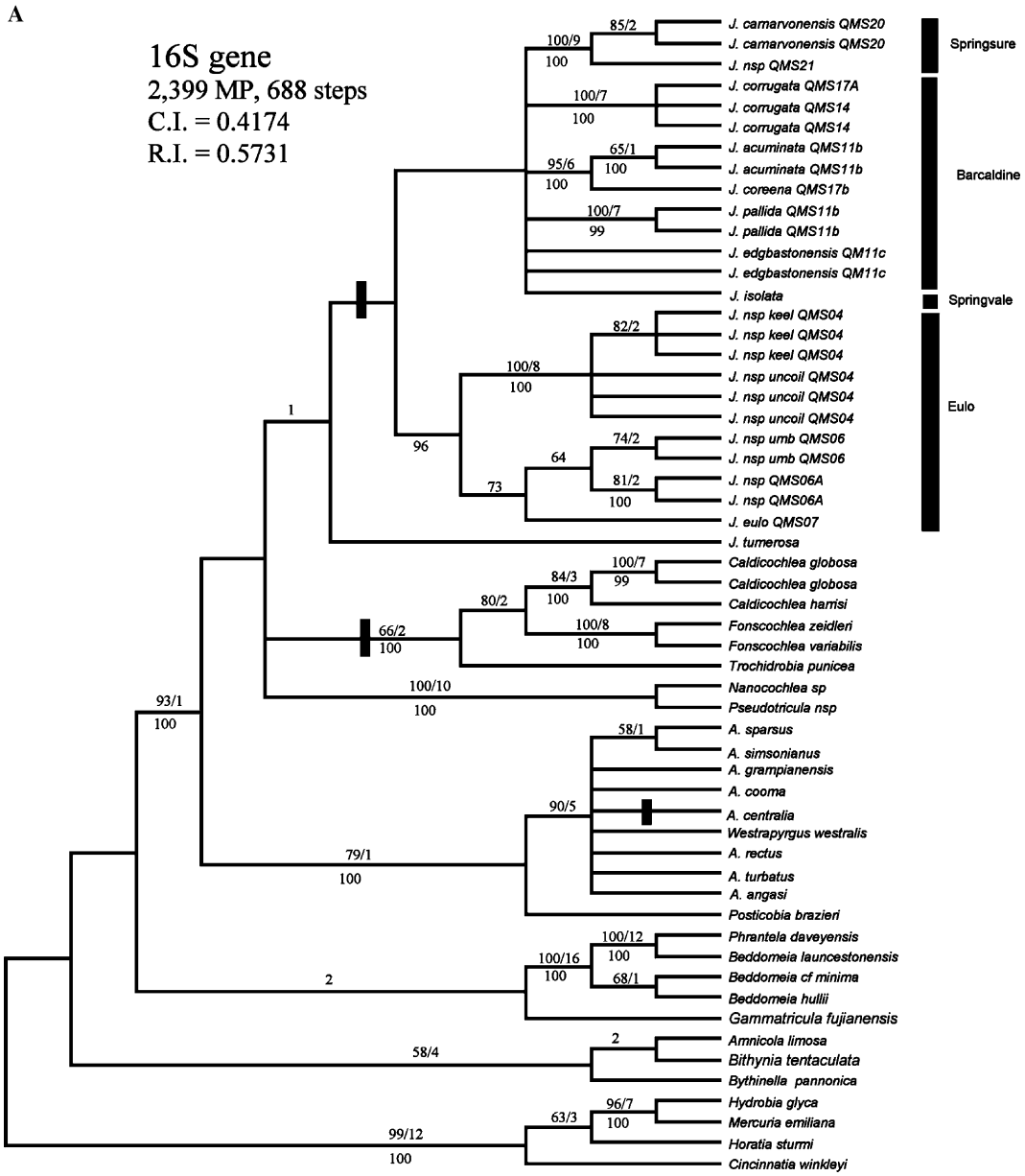

Fig. 2. Strict consensus of the most parsimonious trees found using parsimony analysis of 16S (A) and CO1 (B). Bootstrap support values are above the branches, followed by Bremer's support. Bayesian posterior probabilities are below the branches. Filled hatch-marks indicate Great Artesian Basin lineages, abbreviations: *J*, *Jardinella*; *A*, *Austropyrgus*. A few taxa occur in only one gene tree, 16S: *J*. nsp QMS21, *J. coreena* QMS17b; CO1: *J. jesswisea* QMS11b, *J*. nsp umb QMS04, *J*. nsp QMS05, and *Phrantela marginata.*

parsimony-informative) using maximum parsimony resulted in eight most-parsimonious trees (2037 steps, CI = 0.3814). The results of the Bayesian analysis and a strict consensus of the most parsimonious trees for the combined data are presented in Fig. 3.

Scatterplots of the absolute number of TS and TV against uncorrected genetic distance for 16S and all three codon positions of CO1 were performed (data not shown). Trends revealed by these plots conformed to prior findings for mitochondrial genes (Collins et al., 1996). The absolute number of TS and TV increased linearly as genetic distance increased. TS outnumbered TV among closely related taxa. TS leveled off at 0.15 uncorrected *p*-distance in CO1 and at 0.12 in 16S indicating that saturation of transitions occurred in comparisons beyond this point. Intraspecific sequence differences (uncorrected for multiple hits) ranged from 0–0.01 in 16S and 0–0.07 in CO1. In interspecific comparisons within the ingroup taxa, sequences differed by 0.07–0.01 for 16S, and 0.10–0.15 in CO1. Genetic distance between the focal hydrobiids and other taxa ranged from 0.12 to 0.20 in 16S and 0.20 to 0.23 in CO1. This result indicates that for the ingroup saturation may begin to be an issue among the most distantly related taxa.

3.2. Phylogenetic relationships among the Great Artesian Basin fauna

All analyses recovered similar tree topologies that differed at a few weakly supported internal nodes. For brevity, we present only the trees from the parsimony analysis of individual genes in Fig. 2 and discuss differences from the Bayesian analyses when relevant. In several instances the estimated Bayesian posterior probabilities are higher than bootstrap values. Most

Fig. 2. (*continued*)

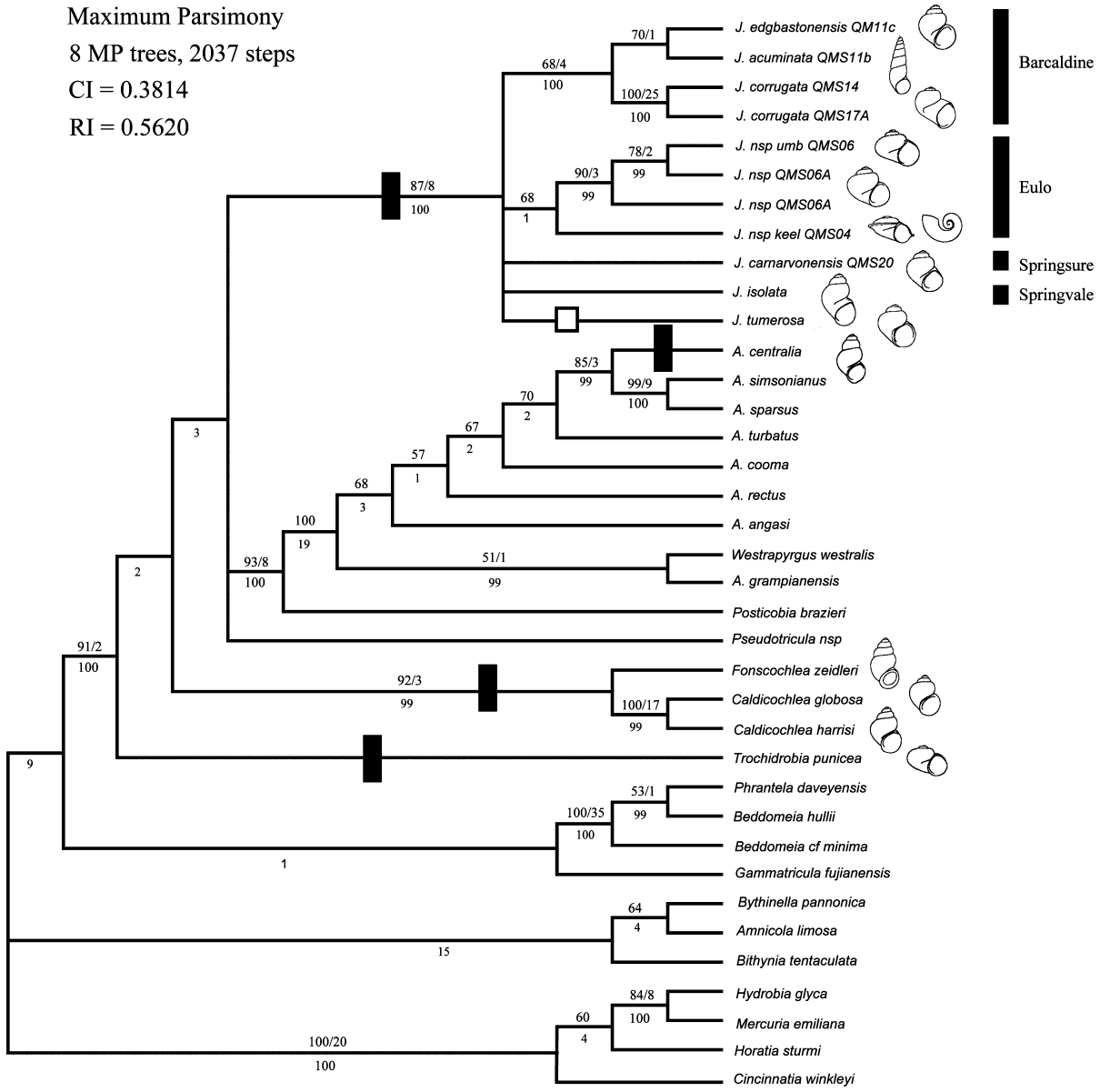

Fig. 3. Strict consensus of the 8 most parsimonious trees of combined CO1 and 16S sequences. Bootstrap support values are above the branches, followed by Bremer's support. Bayesian posterior probabilities are below the branches. Filled hatchmarks indicate the Great Artesian Basin fauna. The hollow hatchmark is *J. tumerosa*, a relative of the GAB fauna but found in a non-GAB Eastern drainage. Abbreviations: *J*, *Jardinella*; *A*, *Austropyrgus*. Shown next to *J.* nsp keeled QMS04 is a closely related (99% sequence identity), uncoiled, new species with a highly divergent morphotype.

are minor differences, such as a node having a bootstrap support of 99 and a posterior probability of 100, however, in several cases bootstrap values in the 50's were estimated as 100% Bayesian support. In cases where there was apparent conflict between bootstrap support and Bayesian posterior probabilities this was assumed to not indicate strong support. Several papers have recently commented on the tendency of Bayesian analyses to overestimate support (Castoe et al., 2004; Cummings et al., 2003) and that trend seems to be supported by this analysis. The combined parsimony tree is shown in Fig. 3.

There were four major clades of Australian taxa in the trees examined, one was composed of the Queensland *Jardinella* species, and the second was a lineage of *Austropyrgus*, *Westrapyrgus*, and *Posticobia*. A third clade is composed of *Fonscochlea* and *Caldicochlea* with *Trochidrobia* included in some trees. *Trochidrobia* is sister to these taxa (comprising the Tateinae) in the CO1 and combined trees. The fourth Australian lineage was composed of *Phrantela* and *Beddomeia* species. None of the analyses supported the monophyly of the GAB fauna and trees constraining GAB monophyly were significantly worse than the most parsimonious trees.

The CO1 analysis included 20 individuals of *Jardinella* encompassing 14 species, including 6 undescribed morphotypes. The 16S sequence analysis included 26 individuals of *Jardinella* representing 15 species, including 6 undescribed morphotypes. The specimens examined were from springs within four different SGs, including the geographically isolated Springvale SG and one individual of *Jardinella tumerosa*, from the non-GAB, Little Mulgrave River, an eastern coastal drainage of Queensland (Fig. 1). Parsimony analysis of 16S sequences resolved *Jardinella* as a monophyletic group. The combined analysis with its more limited sampling found 87% bootstrap support and 100% Bayesian posterior probability for *Jardinella* monophyly; CO1 parsimony and 16S Bayesian analyses did not support *Jardinella* as a monophyletic group or found this clade to be unresolved, however, there is little support for this portion of the tree and all of the most parsimonious trees supported a monophyletic *Jardinella*. An additional analysis was performed on a combined dataset restricted to the spring taxa, excluding taxa that could be affected by saturation effects. This analysis resulted in a single most parsimonious tree (not shown, 1035 steps, CI = 0.4361, HI = 0.5639) entirely congruent with that of the complete combined analysis. This analysis resolved a sister relationship between *Jardinella carnarvonensis* and *Jardinella isolata*, and placed *Jardinella tumerosa* as basal to the rest of the *Jardinella*, however, there was no bootstrap support for either relationship.

For nine species in the 16S analysis more than one individual was sequenced, eight of nine of these were monophyletic units; with the exception of *Jardinella edgbastonensis* whose sister relationship was not resolved, however, one specimen of *J. edgbastonensis* had multiple (~15) N's in the sequence, removal of N's reveals 100% sequence similarity among *J. edgbastonensis* individuals in 16S. In the CO1 analysis, two of the three species with multiple individuals were monophyletic.

The *Jardinella* fauna of each spring SG was usually monophyletic. The Springvale SG contains only a single species, *J. isolata*. Five species from the Barcaldine SG were examined: *Jardinella corrugata*, *Jardinella pallida*, *Jardinella coreena*, *Jardinella acuminata*, and *J. edgbastonensis*. The Springsure SG contained *J. carnarvonensis* and one undescribed species of *Jardinella*, both found within Carnarvon Gorge National Park. The only disagreement among different analyses and genes was in the resolution, or lack of, the Eulo SG as a monophyletic unit. Five species from this group were examined; *Jardinella eulo* and four undescribed species. The parsimony and Bayesian 16S analyses and CO1 Bayesian found the snails in the SG to be monophyletic (with varying support); however, in 16S parsimony analysis this relationship was unresolved. In all analyses two new species with very unusual morphology (shown in Fig. 3) from springs on Bundoona Station were closely related, one of these species is strongly keeled (*J.* sp keel QMS04) and the other is partially uncoiled (*J.* sp uncoil QMS04).

In all trees the *Caldicochlea* + *Fonscochlea* clade was resolved as distinct from the other diverse GAB genus *Jardinella*. *Caldicochlea* individuals form a monophyletic group, and *Caldicochlea globosa* was resolved, but the highly morphologically variable *Caldicochlea harrisi* was not monophyletic. The analyses also support the monophyly of the genus *Fonscochlea* as sister to *Caldicochlea*. The placement of the single representative of the Tasmanian genus *Pseudotricula* varied among analyses; the CO1 parsimony analysis placed *Pseudotricula* as sister to *Austropyrgus*, however, this relationship had weak or no bootstrap support. The Bayesian analysis of CO1 finds *Pseudotricula* basal to the *Jardinella* clade with only 55% support. The 16S parsimony and Bayesian analyses resolved *Pseudotricula* as sister to *Nanocochlea* with 100% support and more closely related to the *Jardinella* + *Caldicochlea* clade than to the *Caldicochlea*. Neither combined analysis provides strong support for the placement of *Pseudotricula*.

3.3. Other Australian hydrobiids

A single species of *Westrapyrgus* from SW Australia was nested within a poorly resolved clade of *Austropyrgus* from SE Australia. In the CO1 and combined analyses *Westrapyrgus* was sister to *A. grampianensis*. None of the analyses found support for the genus *Westrapyrgus* (or, conversely, monophyly of *Austropyrgus*). This clade included one GAB taxon *Austropygrus centralia*, from Dalhousie Springs, rendering the GAB fauna polyphyletic. Analyses constraining monophyly of the GAB fauna were significantly worse than the most parsimonious tree. There was little resolution within the *Austropyrgus* clade. The only well-supported relationship was found in all analyses between *A. simsonianus* and *A. sparsus*. The closest relative of the *Austropyrgus* clade varied: in combined and 16S parsimony and CO1 Bayesian analyses it was *Posticobia*, in the Bayesian analysis of 16S it was *Nanocochlea* + *Pseudotricula*, and in CO1 parsimony it is *Pseudotricula*.

Another Australian lineage is a clade composed of the Tasmanian genera *Phrantela* and *Beddomeia* (Ponder et al., 1993) which forms a monophyletic group with the rest of the Australian taxa in the combined parsimony analysis and 16S parsimony analysis. Relationships of these two genera are unresolved (i.e., Bayesian analysis of 16S, CO1, and combined data) or weakly allied with some of the outgroup taxa (i.e., parsimony analysis of COI data). All analyses reveal some taxonomic uncertainty in *Beddomeia* and *Phrantela*. A specimen of *Phrantela* is well supported in all analyses as being the closest relative to one of the *Beddomeia* species.

4. Discussion

4.1. Phylogeny and biogeography of GAB hydrobiids

It is evident from the mitochondrial gene phylogenies that the GAB hydrobiid radiation is not monophyletic, but is comprised of at least two, possibly three main lineages each representing one or more invasions of the region (see Figs. 2 and 3). One GAB lineage is comprised of the genus *Jardinella* from the Queensland spring SGs. A second GAB lineage is *Caldicochlea* from the Dalhousie SG of northern South Australia and *Fonscochlea* from the Lake Eyre SG. A third GAB lineage, consisting only of *Austropyrgus centralia* in the Dalhousie Springs, is closely related to other members of the genus *Austropyrgus* (genetic distance 16S = 1%; CO1 = 3–5%) as predicted previously (Ponder et al., 1996). This genus is otherwise known from the rivers and streams of southeastern Australia and Tasmania (Clark et al., 2003). The phylogenetic placement of *A. centralia* within the genus in conjunction with its low-level of genetic differentiation suggests that this species is most likely to have reached Dalhousie Springs via dispersal, possibly during the Recent or Pleistocene from nearby suitable habitat such as the Flinders Ranges (Ponder et al., 1996), where extant species of *Austropyrgus* are known to occur (Clark et al., 2003).

Jardinella is the most species rich and morphologically diverse hydrobiid genus found in the GAB springs. Species are found in at least four of the spring SGs and occur along the northern and south-central fringe of the GAB of western Queensland (Ponder and Clark, 1990) (Fig. 2). Three additional non-GAB *Jardinella* species (including the type species of the genus) have been described from the streams and rivers of the coastal slopes of the north Queensland portion of the Great Dividing Range (Ponder, 1991). Although the strict consensus tree based on the mitochondrial CO1 gene does not seem to show *Jardinella* as monophyletic all of the equally parsimonious trees support its monophyly as does the mitochondrial 16S tree and combined analyses. The monophyly of *Jardinella* is supported anatomically by several derived morphological attributes: presence of two basal cusps on the central radular tooth, a white "smear" on the operculum, the shape and position of the seminal receptacle, bursa, coiled oviduct, and the relative size of the albumen and capsule glands (Ponder, 1991; Ponder and Clark, 1990).

Interestingly, the earliest split in the *Jardinella* clade is between *Jardinella tumerosa* (only weakly resolved in 16S parsimony and Bayesian) the non-GAB representative of the riverine eastern seaboard of Queensland, and its sister group, the GAB *Jardinella* of western Queensland. Ponder (1991) hypothesized that eastern seaboard *Jardinella* is derived from the GAB fauna and that it crossed the Great Dividing Range during wet periods of the Pliocene or Pleistocene. This observation was hypothesized, in part, because of the limited morphological divergence observed between the recognized eastern and western taxa (Ponder and Clark, 1990). The deep split observed in the molecular phylogeny between the eastern non-GAB and western GAB fauna does not preclude a dispersal hypothesis; however, it would have likely been an earlier event. Alternatively, the separation of the western GAB and non-GAB fauna may be due to the Early to Middle Tertiary uplift of the Great Divide (Ollier, 1982), which would have separated the two faunas in situ. Fossil hydrobiid snails have been found in non-marine limestones of Miocene age (Ludbrook, 1980; McMichael, 1968), which may add support to a mid-Tertiary origin of the Queensland artesian spring hydrobiid fauna.

Within the GAB *Jardinella* clade, taxa form clusters that correspond largely to the main spring SGs including the Springvale SG (*J. isolata*), Barcaldine SG (*J. corrugata*, *J. pallida*, *J. edgbastonensis*, *J. acuminata*, and *J. coreena*), Springsure SG (*J. carnarvonensis*, *J.* nsp. QMS21), and the Eulo SG (*J. eulo*, plus several newly discovered, undescribed species). The 16S data and combined Bayesian analysis supports the Eulo SG of the south-central fringe of the GAB being sister to the other three SGs, which border the northern and northeastern GAB in CO1 and combined analyses it is unresolved. The position of *J. isolata* varied, 16S analyses place this species as unresolved related to the Barcaldine and Springsure SG's, CO1 analyses have strong support for a sister relationship to Springsure SG taxa, and in the combined parsimony analysis this relationship is unresolved. Combined Bayesian places this taxa basal to Barcaldine SG. Analysis of a limited dataset found a sister relationship between *J. carnarvonensis* and *J. isolata*, and placed *J. tumerosa* as basal to the rest of the *Jardinella*, however, there was no bootstrap support for either relationship. The morphological cladistic analysis of Ponder and Clark (1990) supports *J. isolata* as sister to the rest of the Queensland radiation. Many of the springs found in the Springsure SG flow from sandstones that are part of the same series making up the aquifer of the Great Artesian Basin, however, the water flowing from them actually drains into watersheds east of the Great Divide. One explanation for this puzzling drainage anomaly is perhaps the Springsure SG was disrupted and reoriented by the up-lift of the Great Dividing Range resulting in its drainages flowing to the east instead of the west, due to a change in slope orientation. The up-lift of the Great Divide would also explain the occurrence of the Springsure SG fauna in seeps restricted to the headwaters.

In the South Australian lineage, *C. globosa* and *C. harrisi* of Dalhousie Springs, form the sister group of *Fonscochlea* of the Lake Eyre SG as hypothesized by Ponder et al. (1996) based on morphological evidence.

The sister group to *Fonscochlea* + *Caldicochlea* is uncertain due to the fluctuating position of the clade depending on the gene analyzed or type of analysis used. The 16S analyses place the Tasmanian genus *Pseudotricula* as sister to *Nanocochlea* with 100% support; however, this relationship is contradicted in other analyses. All other analyses have very weak support for other placements of *Pseudotricula*, CO1 parsimony and the combined Bayesian analysis places *Pseudotricula* basal to *Austropyrgus*, CO1 Bayesian places it basal to *Jardinella*, combined parsimony finds an unresolved relationship basal to *Jardinella Austropyrgus*. Other evidence will be required before the exact phylogenetic placement of *Pseudotricula* can be ascertained.

The placement of the conchologically and anatomically unusual GAB genus *Trochidrobia* (Ponder et al., 1989) of the Lake Eyre SG is uncertain. Both COI analyses place this genus as sister to the entire mainland Australian hydrobiid clade, the combined analyses and the 16S analyses place *Trochidrobia* as basal to the *Fonscochlea* + *Caldicochlea* clade, which is biogeographically appealing because it is consistent with the wide overlap of the distribution of the genera in the Lake Eyre and Dalhousie Springs SGs of South Australia. Based on shell and anatomical characters, *Trochidrobia* was thought to be not closely related to any known hydrobiid genus (Ponder et al., 1989). Analyses down-weighting 3rd codon positions in the CO1 analysis give the same result, placing *Trochidrobia* with *Fonscochlea* + *Caldicochlea*. Other evidence will be required before the exact phylogenetic placement of *Trochidrobia* can be ascertained.

4.2. Other Australian hydrobiids

The genus *Westrapyrgus*, of Western Australia, is nested within the genus *Austropyrgus* rendering *Austropyrgus* paraphyletic, although resolution of the relationships within this large group requires more thorough taxonomic sampling. While the current environment associated with the Nullabor Plain and Great Victoria Desert is a major impediment to dispersal, it is possible that during wetter periods in the Miocene and Pliocene (Kemp, 1978) more favorable habitat existed and that the distributions of *Westrapyrgus* and *Austropyrgus* may have been more closely associated. Long-term separation is indicated by significant morphological characters separating these two groups (Ponder et al., 2000). The sister group to *Westrapyrgus* plus *Austropyrgus* is the supposedly monotypic genus *Posticobia*, which is distributed mainly in the coastal streams and rivers of New South Wales and Queensland. This whole clade is characterized by several morphological characters in the female and male reproductive anatomy including the shape and position of the seminal receptacle, and general coiling of the oviduct, the presence of 3–4 basal cusps on the central tooth of the radula and the presence of a smear and two or more pegs on the operculum, which support the monophyly of the three genera (*Posticobia*, *Westrapyrgus*, and *Austropyrgus*).

The molecular phylogenetic data presented herein does not provide support for the monophyly of the Australian hydrobiid fauna. The only analysis which does not reject Australian monophyly is the 16S Bayesian in which these relationships are unresolved. 16S and combined parsimony finds an unsupported relationship of *Gammatricula* with the Tasmanian genera *Phrantela* + *Beddomeia*. CO1 parsimony and Bayesian analyses place *Hydrobia* + *Mercuria* + *Horatia* + *Cincinnatia* within Australian taxa, sister to *Phrantela* + *Beddomeia* with good support. Relationships of Australian hydrobiids with those of the rest of the world are weakly supported in our analyses and somewhat contradictory, further work with worldwide sampling and more conservative genetic markers are needed to resolve these relationships.

Regrettably, the extraordinary fauna of the GAB is imperiled. Most of the Australian spring snails are not in protected areas, but are found on pastoral leases. Heavy usage of artesian water and an ever-increasing demand for water has and will continue to cause extinction of many springs, including their unique fauna (Fensham and Fairfax, 2003; Ponder, 1995, 2004). Without intervention it is only a matter of time before unprotected populations become extinct.

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