

Phylogenetic Analysis of the Hawaiian Damselfly Genus *Megalagrion* (Odonata: Coenagrionidae): Implications for Biogeography, Ecology, and Conservation Biology¹

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ABSTRACT: A phylogeny of the 22 species currently recognized in the genus *Megalagrion*, endemic to the Hawaiian Islands, is presented based on an analysis of 23 morphological and ecological characters. After the exclusion of *M. williamsoni*, known from only a single male, and inclusion of subspecies within their nominate taxa, a single resolved tree of length 85 was obtained; this tree has a consistency index of 0.56 and a retention index of 0.72. Based on this phylogeny, it appears that the major clades within *Megalagrion* differentiated on Kaua'i or an antecedent high island. These clades subsequently colonized the younger islands in the chain in an independent and sequential fashion. The phylogeny also implies an ecological progression from ancestral breeding sites in ponds or slow stream pools to breeding on seeps, with the latter habitat having given rise on one hand to a clade of species breeding in phytotelmata or terrestrially, and on the other hand to a clade breeding in rushing midstream waters. The latter ecological progression also indicates a transformation series in larval gill structure from foliate to saccate and eventually to lanceolate. Most species of current conservation concern are shown to be clustered in particular clades, indicating an inherent phylogenetic vulnerability of certain taxon clusters to novel ecological perturbations; the additional species at risk not present in the above clades are endemics confined to the island of O'ahu and have declined because of their geographic provenance.

THE DAMSELFLY GENUS *Megalagrion* is one of the remarkable endemic products of Hawai'i. The group contains 22 species as currently interpreted, all confined to the high islands of the Hawaiian Archipelago. *Megalagrion* species have long attracted the attention of collectors and amateur naturalists, because they are among the largest and most colorful members of the insular entomofauna. The genus is also of note because of the wide range of larval habits exhibited by its species, with the immature stages occupying habitats ranging from stream pools and wet rock faces to phytotelmata and damp leaf litter. The last revision of the group was

that of Zimmerman (1948) in vol. 2 of *Insects of Hawaii*, which recognized 22 species, plus five subspecies (independent of the nominate subspecies). An additional species, *M. paludicola*, was described from Kaua'i by Maciolek & Howarth (1979), who also reviewed the taxonomic history of the genus. It is worth noting that of the 22 species recognized herein, 21 were described before 1900, primarily from the material collected by R. C. L. Perkins, which formed the basis for *Fauna Hawaiiensis* (Perkins 1899, 1910). During the current century, however, many of the species in the genus have become very scarce, with the lowland species in particular having become locally extirpated on certain islands. As a result, six species have now been proposed for listing under the Endangered Species Act (U.S. Fish and Wildlife Service 1994). An extensive discussion of the historical factors that placed these *Megalagrion* species at risk, and an evaluation of the current status of individ-

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ual species, can be found in Polhemus (1993, 1996) and Polhemus and Asquith (1996).

Characters

The primary adult character systems used as a basis for the current phylogenetic hypothesis were the shape of the labium, the structure of the mesostigmal lamellae of the females, and the structure of the abdominal terminalia in males. Morphological terms for the female mesostigmal structures follow Garrison (1984) insofar as is possible. Certain additional mesostigmal structures not utilized by Garrison were found to be useful in species separation and are assigned names herein. For the structures of the male terminal abdomen the terms "superior appendage" and "inferior appendage" are retained to avoid confusion among nonspecialists who might use the present work in combination with previous treatments of the group, notably that of Zimmerman (1948). Technically, the superior appendage as treated here is the cercus, and the inferior appendage is the paraproct. As with the female mesostigmal structures, certain discrete features of these male appendages were found useful in species separation and are assigned names herein.

The primary immature characters utilized were the shape of the prementum and, most important, the shape of the gills. The latter character is linked to breeding ecology and varies distinctively among species in the genus.

A single ecological character, breeding habitat, was also utilized, because it varied markedly among species and clades, and appeared to offer useful phylogenetic information.

The various morphological character systems are discussed in greater detail below and will be illustrated in a forthcoming monograph of the genus (unpubl. data).

ADULT MALE ABDOMINAL APPENDAGES. The terminal appendages of the male abdomen provide excellent characters for the separation of all *Megalagrion* species. The superior appendage (cercus) shows a large degree of modification among various species, but almost uniformly retains several common elements. These include a stout, expanded *basal section*, which is often produced along its inner margin into a rounded *basal flange*. This flange often bears one or more

inner projections, which in certain species may be elongated and folded upward. This basal section of the superior appendage narrows to form an elongate *distal arm* with a modified *grasping pad* at its tip. The shape of this pad is conserved in nearly all species (except *M. xanthomelas* and *M. oahuense*, where it is highly modified) and provides a useful morphological marker by which one may assess the relative length of the distal arm in various species. In many species there is also a *ventral projection* on the distal half of the appendage, giving the tip of the appendage a bifurcate appearance.

The inferior appendage (paraproct) is much more conservative in its degree of structural variation, and in most species has a tapering, falciform shape when viewed laterally. The degree of prolongation is variable, as is the degree of upward curvature, this latter being most pronounced in *M. paludicola*. In a few species the appendage has a *bifurcate tip* (i.e., *nigrohama-tum* clade), and in several others (*M. pacificum*, *M. xanthomelas*) there is a *subapical tooth* on the dorsal margin.

ADULT FEMALE MESOSTIGMAL LAMELLAE. In contrast to the situation in many other genera of Zygoptera, the female mesostigmal lamellae of *Megalagrion* provide excellent characters that allow the unambiguous discrimination of individual species. With the exception of a few structural elements, there seems to be no consistent terminology that has been applied to the various components of these lamellae, and as a result certain new terms are introduced herein. The central section of the lamellae is dominated by a tongue-like structure called the *ramus*, which arises from a *central depression* below a *central transverse bridge*. The shape of this bridge varies from rectangular to trapezoidal and is distinctive for certain clades within the genus. Flanking the ramus and bridge to either side anterolaterally are a pair (1+1) of large *membranous depressions* with raised margins; these are often roughly triangular, but in certain species may be greatly expanded and rounded posteriorly (as in the *blackburni* clade). Within these depressions, near the inner margin, is a small, rounded *membranous lobe* that is vaguely reminiscent of a pineapple and varies in shape among species.

The posterior margin of each membranous depression is defined by a *posterior transverse*

member that lies perpendicular to the body midline; this member is generally well developed, has various degrees of setiferation among species, and may often be produced into an overhanging lip laterally. Behind this posterior transverse member there is usually a *postlamellar depression*, which in most species is transverse and parallel to the member, but in some species is highly produced posteriorly and roughly ear-shaped (e.g., *calliphya* clade). In cases where the depression is transverse, it is occasionally flanked posteriorly by a *mesepisternal tumescence*, leaving the depression as a deep groove between the lamellae and the tumescence (this is particularly well illustrated in *M. nesiotus*).

The anterolateral margins of the membranous depression are defined by a smooth, glabrous *anterolateral member*, which is little modified across the genus, although in certain species it may bear a weak transverse depression centrally. The anterior margins of the membranous depression are formed by a set of ridged and folded membranes that seem to offer few useful characters for phylogenetic reconstruction.

ADULT LABIUM. The shape of the adult labium is distinctive among various *Megalagrion* species. In particular, the shape of the medial cleft may vary from narrow and slitlike to broad and U-shaped, and the shapes of the flanking lateral lobes may vary similarly in terms of their width and apical curvature.

IMMATURE GILLS. Three main gill types are present in the immatures of *Megalagrion* species: foliate, saccate, and lanceolate.

Foliate gills, which are common across the Coenagrionidae and appear to be the ancestral state in *Megalagrion*, are broad, ovate, and flattened, with an extensive network of branching veins internally (see Figure 8). In certain species, such as those of the *calliphya* clade, these gills are divided into two distinct parts, a heavily pigmented basal section and a lightly pigmented distal section. The tips of such gills also show variation in their degree of prolongation, being extremely elongated in the species of the *nigrohamatum* clade.

Saccate gills, which are seen in all those species that breed in phytotelmata plus certain species that breed on seeps, are short, stout, and inflated (see Figure 8). In cross section the two

lateral gills are triangular and the medial gill is diamond-shaped. This allows the medial gill to be cradled between the lateral gills when the larva is resting against the flat surface of a leaf. One should note that the term "saccate" as used herein to refer to gill structure is not exactly equivalent to "saccoid" in the sense of Tillyard (1917), because he considered the saccate gills of Hawaiian species such as *M. koelense* to be intermediate between his "triquetro-quadrata" and "saccoid" types. Even so, these gills are inflated and thus saclike, and the term "saccate" thus seems reasonable when used regionally within the context of the Hawaiian fauna.

Lanceolate gills are long, tapering, and blade-like, and are triangular in cross section basally (see Figure 8). Gills of this type are seen in certain seep-breeding species and in all species that breed in swift, midstream waters. The margins of such gills are usually serrate and often bear modified, spinelike setae.

IMMATURE PREMENTUM. The shape of the immature prementum in *Megalagrion* is generally trapezoidal, as is typical across Zygoptera as a whole. In the *nigrohamatum* clade, however, this structure has been modified to a lunate shape, which provides a useful synapomorphy uniting the species within this group.

Origins and Outgroups

The insular radiation of damselflies seen on the Hawaiian Islands is far removed from that of any other similar endemic cluster in the Pacific Basin, and the source area for this Hawaiian colonization remains an open question. The damselfly fauna of the insular Pacific islands east of the Tonga Trench and the Marianas is composed exclusively of species in the family Coenagrionidae, with four genera represented: *Ischnura*, *Pseudagrion*, *Coenagrion*, and *Teinobasis*. It thus seems logical that one of these lineages may have provided the original dispersing colonists that gave rise to *Megalagrion*.

Ischnura species are distributed throughout Polynesia and Micronesia, except for Hawai'i (although two exotic species have now become established in the latter area through human commerce). Most of this vast Pacific distribution is dominated by a single tramp species, *Ischnura aurora*, but local endemics are present on Tahiti,

Rapa, and Bora Bora–Raiatea, and a local radiation of five species has arisen on Samoa. Although highly dispersive and therefore a logical candidate to reach Hawai'i, no native species of *Ischnura* occurs in the Islands. In addition, *Ischnura* is far removed morphologically from *Megalagrion* and therefore an unlikely outgroup candidate on phylogenetic grounds.

Zimmerman (1948) considered *Megalagrion* to be closely allied to *Pseudagrion*, a genus that is highly diversified in the Palearctic region, but which occurs in the Pacific only as single scattered endemic taxa on Fiji, Samoa, Palau, and the Marquesas, with no apparent representation on any intervening island group. This is a most illogical distribution, with only one highly disjunct species east of the Tongas and Marianas, and suggests that the Pacific species currently grouped within *Pseudagrion* may represent a polyphyletic assemblage. In particular, *P. demorsum* Needham from the Marquesas appears to be morphologically aberrant and may represent a separate generic entity whose sister-group relationships are not well established. No correlation between larvae and adults has been established for any Marquesan damselfly species, but Needham (1933) published an illustration of an immature taken from "under stones in a stream bed" that has a gross morphology extremely similar to that of *Megalagrion blackburni* from Hawai'i. This strongly suggests that a sister-group relationship may exist between certain elements of the damselfly faunas in these two archipelagoes.

The genus *Coenagrion* is a predominantly north-temperate genus, with limited representation in Africa, South America, tropical Asia, and Australia. Two species are known from Polynesia, the poorly understood *C. melanoproctum* Selys from "Polynesia," and *C. interruptum* from the Marquesas. As in *Pseudagrion*, these Polynesian representatives are doubtfully congeneric with the remainder of the genus, with Needham (1932) having noted of his *C. interruptum* that "This species does not quite fit *Coenagrion*, and another new genus might with abundant precedents be erected for it, were it not that there are already too many undefinable genera in this part of the series." Needham's comments are indicative of the confusion still extant within the classification of insular Pacific

damselflies and the consequent difficulties of accurately assessing outgroups for *Megalagrion*.

In contrast to the two preceding genera, *Teinobasis* is absent from Polynesia, having instead a Micronesian distribution, with single-island endemics on Palau and Truk, and a radiation of five insular endemics on Ponape. One of the Ponape endemics, *T. ariel*, is known to breed in the leaf axils of *Freycinetia* in a manner identical to that of several *Megalagrion* species on Hawai'i, and the gross morphology of the immatures in both cases is quite similar. The phytotelmata breeding habit could be extremely advantageous in terms of dispersal, because it would allow a species to colonize islands where streams and ponds were not available.

For the purposes of this analysis, the genus *Pseudagrion* was selected as the outgroup based on its general overall morphological similarity to *Megalagrion* in both adult and immature stages. Character states were analyzed for *Pseudagrion civicum* Lieftinck from New Guinea, for which recently collected specimens of both adults and immatures were available. Future analyses will add additional outgroups, including Micronesian and Marquesan taxa, to assess more fully the phylogenetic affinities of *Megalagrion* in an overall Pacific context.

Phylogeny

Morphological and ecological data were analyzed to obtain a set of characters that appeared useful in determining the phylogeny of *Megalagrion* species. These characters came from four major sources: adult females, adult males, immatures, and ecology. The characters in the first two sets were analyzed primarily by use of scanning electron micrographs, which sharply illustrated many previously undescribed structures. Immature characters were analyzed primarily by light microscopy using cast exuviae or alcohol-preserved specimens. Ecological characters were based on 5 yr worth of field observations and collecting, plus published life history notes from the literature.

Twenty-three characters were identified that offered useful phylogenetic information. These characters and their states are described below. The character states are coded using integers beginning with 0; many of these characters were

coded as multistate, with the number of discrete states for a given character varying from 2 to 6 (see Table 1). The character numbering indicates no a priori assumptions as to character state polarity.

Descriptions of Characters and Character States for Megalagrion Species

Character states marked with an asterisk (*) indicate the state present in the outgroup, *Pseudagrion civicum* from New Guinea.

ADULT FEMALES

1. Medial cleft of labium: 0 = narrow and slitlike; 1 = broad with rounded posterior margin*; 2 = broad with angulate posterior margin.
2. Lateral lobes of labium: 0 = very broad; 1 = broad with rounded apices; 2 = broad with angulate apices; 3 = slender and acuminate*.
3. Mesepisternal tumescence behind mesostigmal lamellae: 0 = absent*; 1 = present.

4. Ear-shaped carina produced posteriorly behind posterior transverse member of female mesostigmal lamellae: 0 = absent*; 1 = present.

5. Setae on posterior transverse member of female mesostigmal lamellae: 0 = absent*; 1 = short, often bristlelike; 2 = long and slender.

6. Central brace of female mesostigmal lamellae: 0 = transversely rectangular*; 1 = narrowed and trapezoidal.

7. Upper portion of ramus: 0 = V-shaped*; 1 = expanded.

8. Posterolateral margin of posterior transverse member on female mesostigmal lamella: 0 = vertical to weakly concave*; 1 = strongly concave and overhanging posteriorly.

9. Depressed channel behind posterior transverse member on female mesostigmal lamella: 0 = absent*; 1 = narrow and weakly defined; 2 = broad and cuplike, curving posteriorly adjacent to ramus; 3 = deep and transverse, orientation roughly perpendicular to ramus.

TABLE 1
CHARACTER STATE MATRIX FOR *Megalagrion* SPECIES

TAXON	CHARACTER NUMBER			
	Females 1234567890	Males 111111 123456	Larvae 11122 78901	Ecology 2 2
<i>Pseudagrion</i>	1300000000	131040	00210	3
<i>adytum</i>	2300100010	121020	23102	4
<i>blackburni</i>	0000110011	011014	23110	2
<i>calliphya</i>	1301001121	111003	01000	3
<i>eudytum</i>	2200100011	121132	23201	4
<i>hawaiiense</i>	1100100010	121011	23101	4
<i>hawaiiense</i> (Kauai)	2200100010	121011	?????	?
<i>heterogamias</i>	1100110012	111014	23110	2
<i>jugorum</i>	2210100030	221031	?????	?
<i>kauaiense</i>	2100100010	121024	13100	5
<i>koelense</i>	1200100010	221121	13100	5
<i>leptodemias</i>	2301001221	111003	01000	3
<i>molokaiense</i>	1110100030	221030	?????	?
<i>nesiotes</i>	1110200030	221131	?????	6
<i>n. nigrohamatum</i>	1300000120	001003	02000	3
<i>n. nigrolineatum</i>	1300000120	001003	02000	3
<i>oahuense</i>	1110200010	221020	13102	6
<i>oceanicum</i>	1200010011	111010	23110	2
<i>oresitrophum</i>	2201001120	011003	01000	3
<i>orobates</i>	0300000120	001003	02000	3
<i>pacificum</i>	0200100110	020000	02000	3
<i>paludicola</i>	2200100010	121011	23201	1
<i>vagabundum</i>	0100100010	121002	13200	4
<i>williamsoni</i>	????1???10	221020	?????	?
<i>xanthomelas</i>	2200100122	020043	00000	0

10. Anterior margin of posterior transverse member of female mesostigmal lamellae: 0 = straight*; 1 = weakly sinuate; 2 = strongly sinuate, produced posteriorly adjacent to ramus.

ADULT MALES

11. Length of inferior appendages: 0 = long and slender, surpassing superior appendages; 1 = slender and falciform, but equal to or less than length of superior appendages*; 2 = short and semitriangular.

12. Tip of inferior appendages: 0 = bifid; 1 = cupped; 2 = simple and acuminate; 3 = simple and blunt*.

13. Subapical tooth on inferior appendages: 0 = present; 1 = absent*.

14. Development of pruinosity on thorax: 0 = absent*; 1 = present.

15. Subapical projection on ventral margin of superior appendage: 0 = absent; 1 = sharply pointed; 2 = cuplike; 3 = small and toothlike; 4 = broad and flattened*.

16. Interior flange of superior appendage: 0 = barely developed*; 1 = forming a broad semicircular interior flange without teeth or projections; 2 = forming a broad semicircular interior flange bearing a small, triangular tooth near center of inner margin; 3 = forming a broad interior flange with the inner margin curved upward to form a fingerlike projection; 4 = forming a broad interior flange with the inner margin curved upward to form multiple fingerlike projections.

IMMATURES

17. Gill type: 0 = lamellate*; 1 = saccate; 2 = lanceolate.

18. Shape of gill tip: 0 = rounded*; 1 = rounded with small point at extreme apex; 2 = rounded with prolonged point at extreme apex; 3 = sharply acute.

19. Gill ridges: 0 = absent; 1 = present with smooth margins; 2 = present with crenate margins*.

20. Spines on gill ridges: 0 = absent; 1 = present*.

21. Gill setiferation: 0 = lacking setiferation*; 1 = covered with scattered short stiff hairs; 2 = densely covered with long pilose hairs.

22. Shape of immature labium: 0 = trapezoidal, angulate*; 1 = expanded and lunate.

ECOLOGY

23. Breeding habitat: 0 = lowland ponds and wetlands; 1 = upland swamps; 2 = perennial streams, erosional zones (= riffles); 3 = perennial streams, depositional zones (= pools)*; 4 = rheocrenes; 5 = phytotelmata; 6 = terrestrial.

Computer Analysis

The characters described above were coded in a matrix and analyzed using the computer program PAUP 3.1.1 (Swofford 1993). The initial analysis was performed on 25 taxa, including the subspecies *M. nigrohamatum nigrolineatum*, and the Kaua'i populations of *M. hawaiiense*, which Daigle (in lit.) has hypothesized are a distinct species. The initial computer run used a Heuristic search with 100 repetitions of random stepwise addition sequences, which discovers "islands" of shortest trees that may not be detected by other addition sequences. The ingroup (*Megalagrion*) was constrained to be monophyletic in relation to the outgroup (*Pseudagrion*), and ambiguous characters were optimized using the accelerated transformation (ACCTRAN), which assumes single origins for characters followed by subsequent reversals (versus the DELTRAN delayed transformation, which assumes multiple origins for similar states). All characters were run unordered.

This initial analysis produced 10 trees of length 85 (i.e., the number of steps required to fit the data to the tree [Figure 1]). A strict consensus tree computed from these 10 trees (Figure 2) revealed that three major unresolved nodes were present:

(1) At the base of a large clade containing *M. williamsoni*, *M. oahuense*, *M. koelense*, *M. molokaiense*, *M. nesiotese*, and *M. jugorum*.

(2) At the base of the clade containing both segregates of *M. hawaiiense* plus *M. eudytum* and *M. paludicola*.

(3) At the base of a terminal clade containing the subspecies of *M. nigrohamatum* plus *M. orobates*.

The polytomy in the first case was clearly caused by the inclusion of *M. williamsoni*, which is known only from a single male and could therefore not be scored for characters from females, immatures, or ecology. The large number of question marks in the matrix resulting

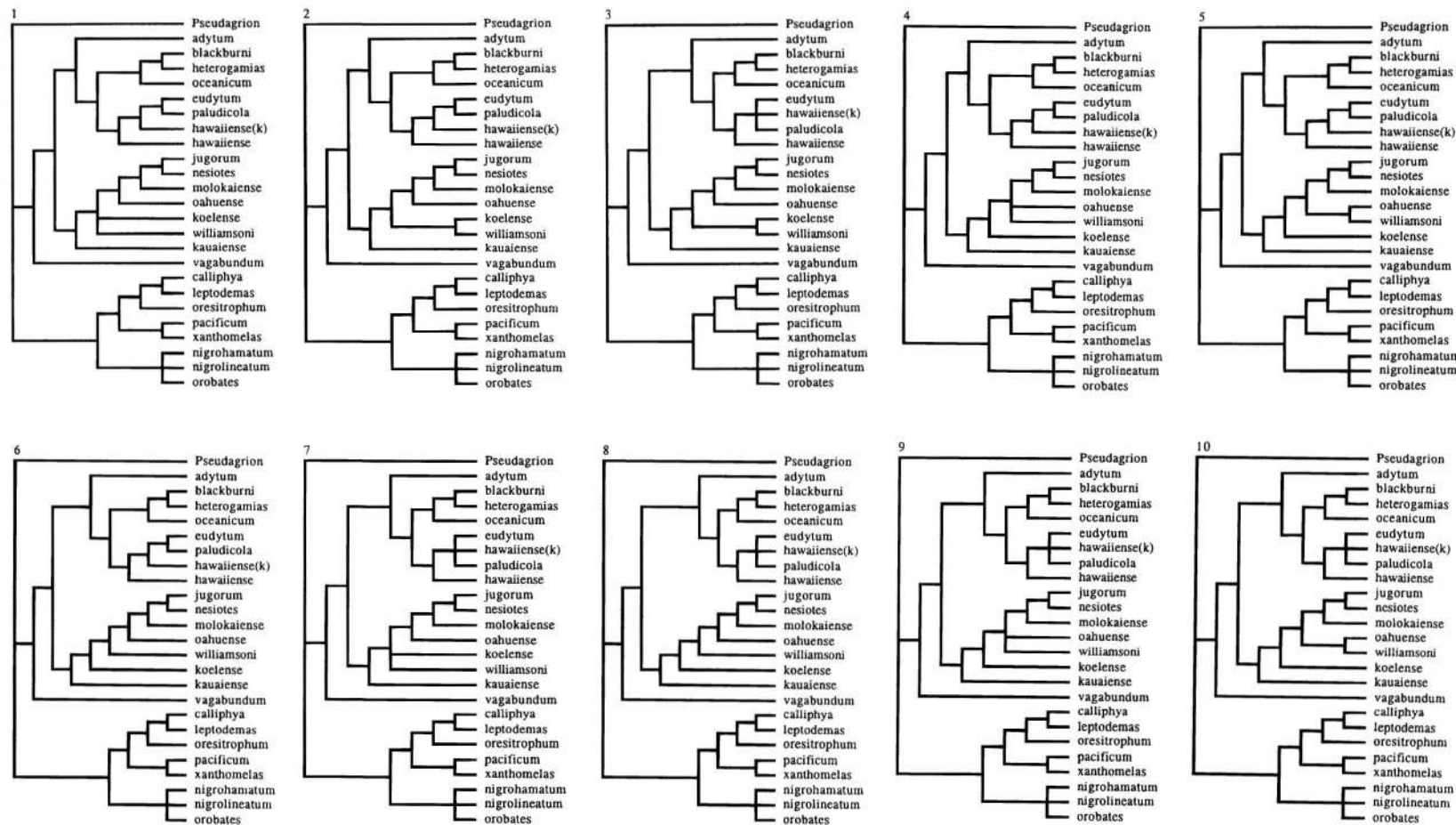


FIGURE 1. Ten shortest trees from initial phylogenetic analysis, with *M. williamsoni* and intraspecific taxa included.

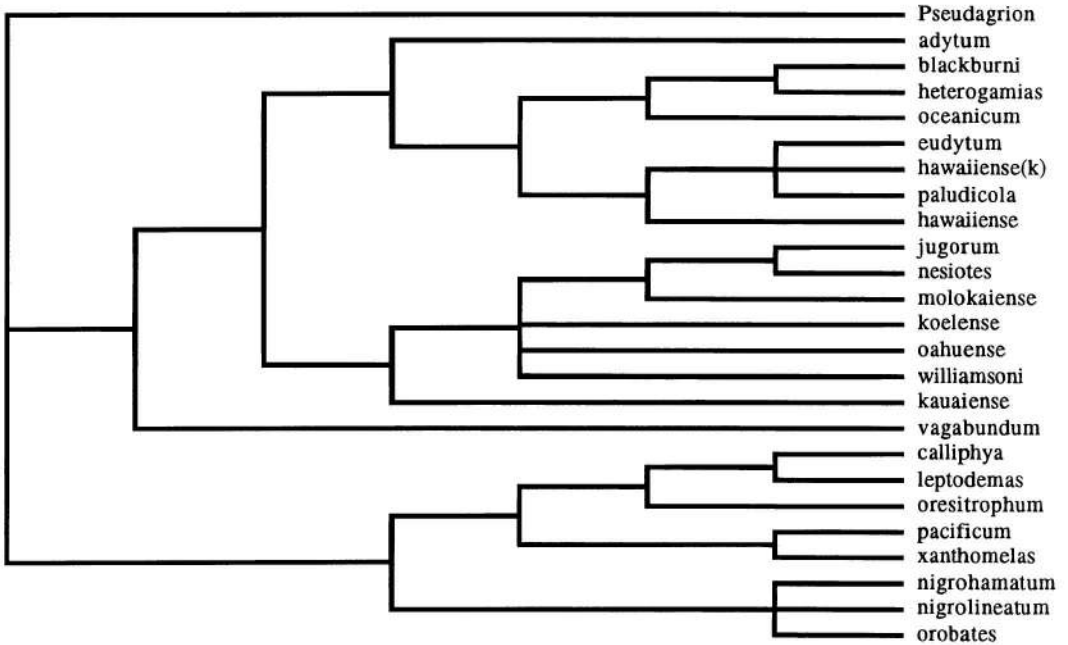


FIGURE 2. Strict consensus tree derived from the 10 trees presented in Figure 1.

from this missing character state information made it impossible for the program to adequately constrain the terminal location of *M. williamsoni*, although all the shortest trees clearly indicated that this taxon was a member of the *oahuense* clade. Because its clade placement had been determined through the initial analysis, it was decided to delete *M. williamsoni* and run the analysis once again. This produced two trees of length 85, with complete resolution of the clade from which *M. williamsoni* had been removed (Figure 3), but with polytomies remaining on two other branches.

These remaining polytomies still involved the intraspecific segregates of *M. hawaiiense* (*M. hawaiiense* from Kaua'i versus *M. hawaiiense* populations from all other islands), and *M. nigrohamatum* (the subspecies *M. nigrohamatum nigrohamatum* and *M. nigrohamatum nigrolineatum*). In the former case information for breeding habit and larval morphology is unknown for the Kaua'i populations of *M. hawaiiense*, leading to numerous undefined character states, and the two characters that scored differently between the two putative taxa both involve nuances of labium shape that may

be attributable to intraspecific variation. In the latter case each member of the *nigrohamatum* pair had coded identically for all character states, because the subspecies definition is based primarily on geographic provenance (O'ahu versus Maui Nui) and adult coloration, characters that are not utilized in the current matrix. Because the initial analyses had demonstrated that each of these taxon pairs were sisters within their respective clades, it was clear that the differences between them were minor and likely did not justify species-level separation, so it was decided to collapse them into single nominate species in each case. Running the analysis with this reduced set of 22 taxa produced a single completely resolved tree with a length of 85, a consistency index of 0.56, and a retention index of 0.72. This tree is presented in Figure 4 and forms the basis for the subsequent discussions of ecological and character evolution within the genus.

Because ecological characters were used in the matrix and might correlate with certain morphological characters (i.e., gill structure), an analysis was also performed excluding the ecological characters from the analysis. This pro-

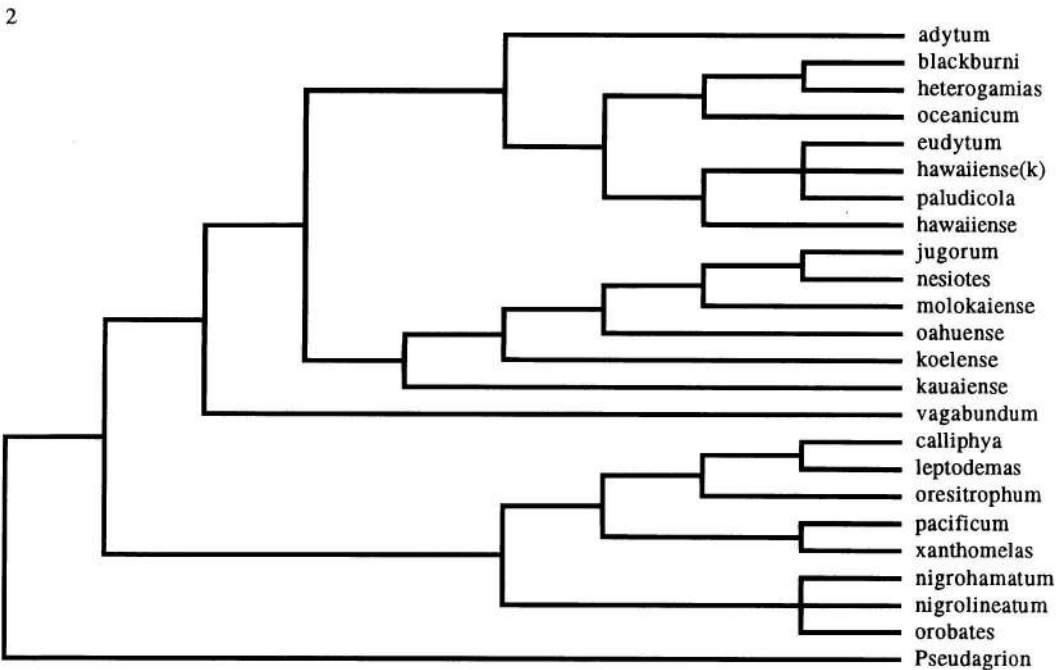
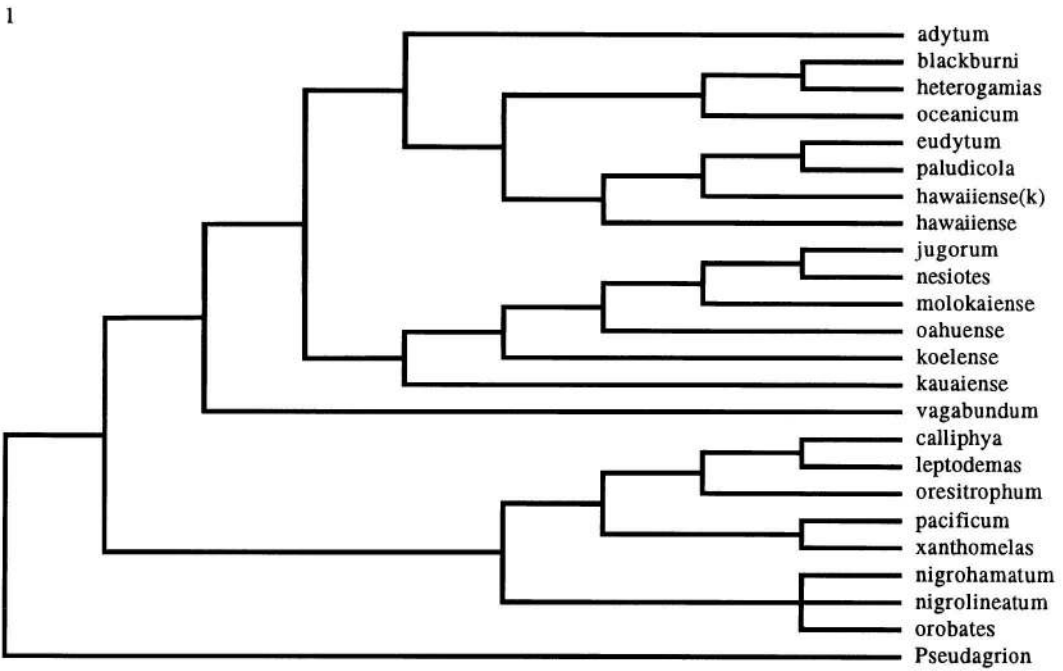


FIGURE 3. Two shortest trees resulting from the revised phylogenetic analysis with *M. williamsoni* deleted from the data matrix (for explanation see text).

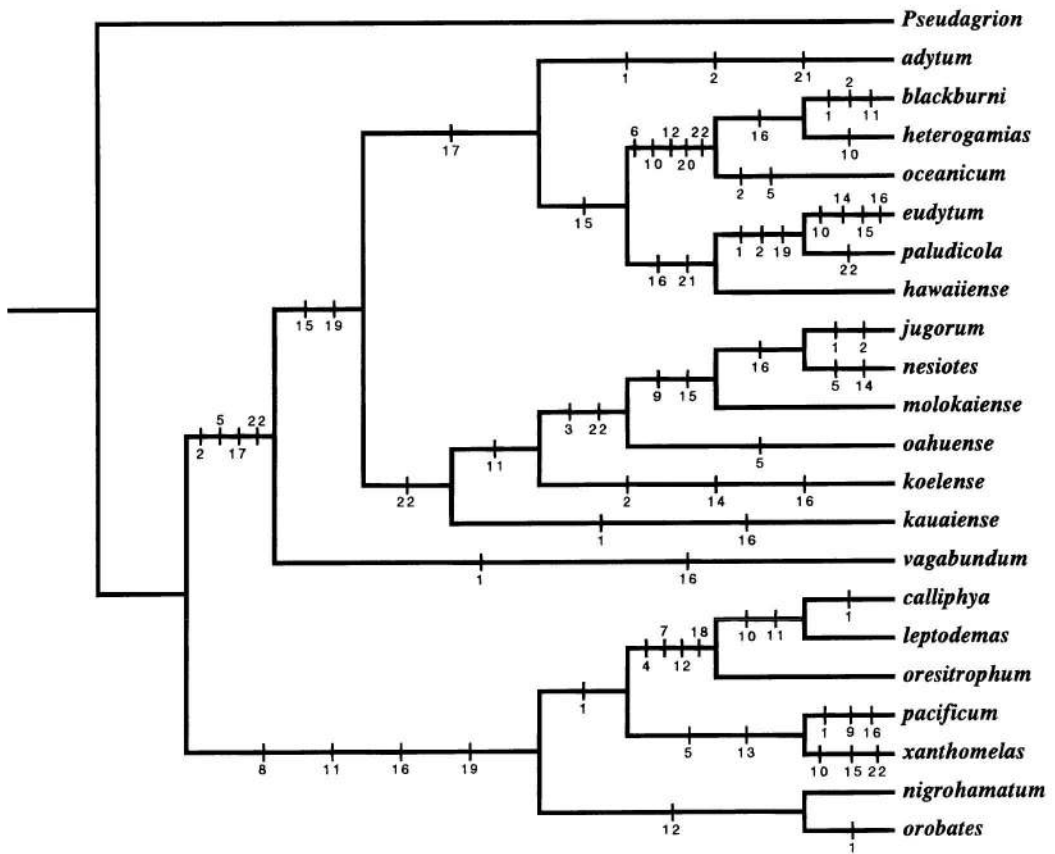


FIGURE 4. Single most parsimonious tree produced after the elimination of *M. williamsoni* and intraspecific taxa from the data matrix (for explanation see text). Numbers and tick marks indicate characters changing state along a given branch. Tree diagnostics are as follows: L = 85, CI = 0.56, RI = 0.72.

duced five shortest trees of length 79, each with a consistency index of 0.532 and a retention index of 0.704. The structure of these trees was identical to that of the trees derived using the full matrix, except that the positions of *M. adytum* and *M. kauaiense* were less constrained in relation to the *oahuense* and *blackburni* clades, with *M. kauaiense* being placed either as the sister taxon to *M. adytum* or in a basal position relative to a clade containing *M. adytum* plus the *blackburni* and *hawaiiense* clades. This result, in combination with the relatively slight decrease in the retention and consistency indices, indicated that the inclusion of ecological characters was not having a disproportionate effect on the overall tree structure and instead simply served

to better define the positions of a few individual species.

Discussion

BIOGEOGRAPHY. The biogeographic distributions of the various *Megalagrion* species are plotted on the cladogram in Figure 5. For the purposes of this analysis the islands composing Maui Nui (Maui, Moloka'i, Lāna'i, and Kaho'olawe) were combined with Hawai'i Island, because the latter island supports no *Megalagrion* species that are not also shared with the former area. An examination of this biogeographic tree immediately reveals that all of the major clades are present on Kaua'i and that this

BIOGEOGRAPHY

■ outgroup

■ KAUAI

▨ OAHU

▩ MAUI NUI & HAWAII

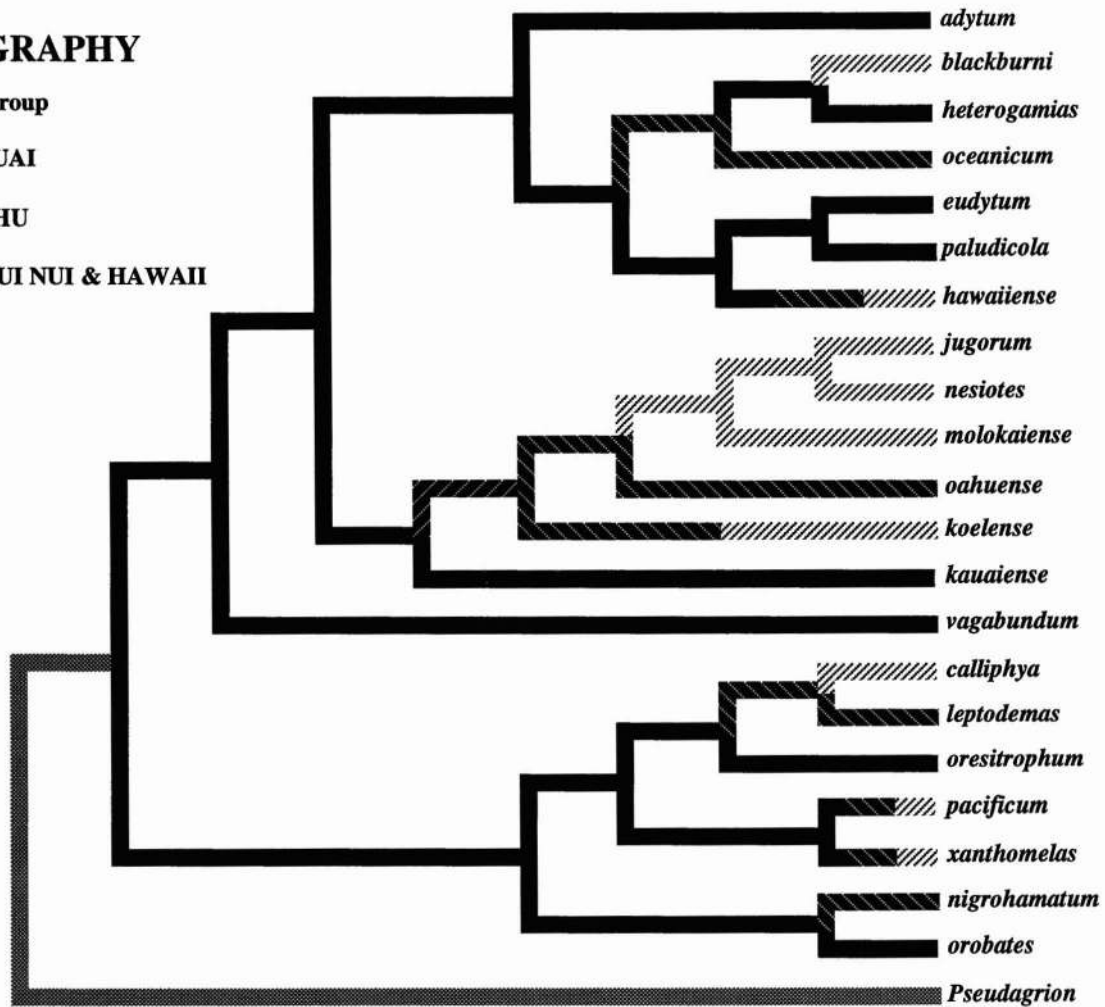


FIGURE 5. Taxon-area cladogram for *Megalagrion* species, with branches shaded to indicate biogeographic areas occupied by various species.

area can be traced to all deep nodes, indicating an initial diversification of the genus on that island or a predecessor and thus a minimum age for these clades of at least 5.5 million yr.

If one looks at the tree in terms of three area statements, one finds a repeated pattern wherein Kaua'i is the basal sister area to O'ahu plus Maui Nui and Hawai'i combined. This is completely logical in terms of the geological formation of the island chain and most likely reflects sequential colonization of newly created islands by the various clades. The only instance in which this pattern is not repeated is in the *blackburni* clade, where O'ahu is basal to Kaua'i plus Maui Nui and the Big Island. This indicates that the *blackburni* clade may have arisen on O'ahu, then radiated back to Kaua'i, then spread from Kaua'i to the younger islands beyond O'ahu as they were formed over the last 2 million yr. Conversely, the current pattern could also have been produced by the extinction of an ancestral Kaua'i endemic within the *blackburni* clade.

BREEDING ECOLOGY. As is typical of isolated island biotas, *Megalagrion* species have radiated into a wide range of specialized breeding habitats, as discussed in detail by Polhemus and Asquith (1996). The distribution of these various breeding ecologies is plotted on the cladogram in Figure 6. This analysis indicates that the ancestral state, present in *Pseudagrion*, is one in which species breed in ponds and stream pools, as is seen in all members of the *xanthomelas*, *calliphya*, and *nigrohamatum* clades. The next ecological condition encountered on the tree is one in which the immatures occupy seeps and other moist, semiterrestrial habitats. This seep-breeding habit then gives rise to two separate lines of ecological specialization. In the *blackburni* clade, all members live in the benthos of swift streams, amid fast-moving waters. In the *oahuense* clade, by contrast, the basal members breed in phytotelmata, and the more terminal taxa breed terrestrially in damp leaf litter beneath fern banks or have ecologies that are currently unknown.

The general progression of breeding ecologies on the tree is from slow water to seeps to fast water, or from slow water to seeps to phytotelmata to terrestrial. The only exception to these trends is seen in *M. paludicola*, a Kaua'i endemic that breeds in still, dark pools amid

upland bogs, but occurs within a clade whose other members are all either seep or fast-water breeders; this breeding strategy is thus interpreted as a reversal to a more plesiomorphic ecology, although these acidic upland pools in fact represent a very specialized habitat. In combination with the biogeographic analysis, one may further hypothesize that the transition from seep breeding to fast-water breeding may have occurred on O'ahu, because the most primitive member of the fast-water clade is present on that island (see previous discussion under Biogeography).

Based on the ecological cladogram, it is also possible to make an informed hypothesis regarding the breeding ecologies of those species for which this information is still unknown. It seems likely, given their terminal position in the *oahuense* clade, that *M. jugorum* and *M. molo-kaiense* will prove to have terrestrial breeding ecologies similar to that of *M. oahuense*. This has important implications in terms of both survey protocols and conservation strategies for these extremely rare species.

EVOLUTION OF GILL TYPE. Three major types of gills, correlated with larval breeding ecology, are represented among the larvae of *Megalagrion* species: foliate, saccate, and lanceolate. The distribution of these gill types is plotted on the cladogram in Figure 7. Foliate gills are found among those species breeding in ponds or slowly moving stream pools, saccate gills are found among those species breeding terrestrially or in phytotelmata and in certain species breeding on seeps, and lanceolate gills are found among other species breeding on seeps and in all species breeding in the midstream benthos amid fast waters. The phylogeny implies an obvious evolutionary progression from foliate through saccate to lanceolate (Figure 8). This once again indicates an ecological transition from slow-water breeding to seep breeding and then subsequently to fast-water breeding. Thus it appears that the *Megalagrion* ancestor that initially colonized the Islands was a slow-water breeder with foliate gills. This also implies that species left the slow reaches of streams for a semiterrestrial existence on seeps and then reinvaded the fast reaches of streams after they had acquired the necessary morphological specializations resulting from a seep-breeding exist-

BREEDING ECOLOGY

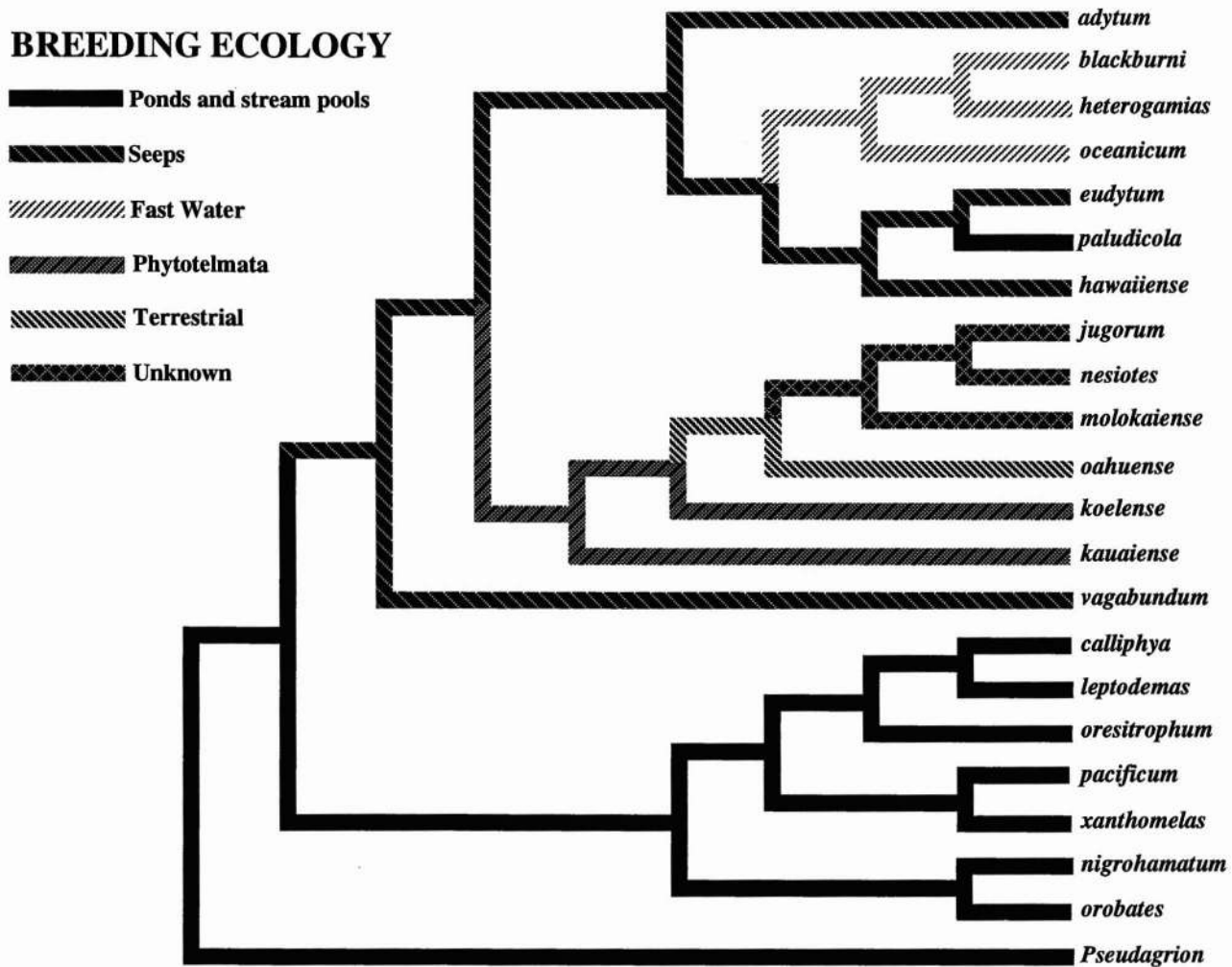


FIGURE 6. Breeding ecology of *Megalagrion* species in relation to phylogeny, with branches shaded to indicate breeding ecologies of individual species.

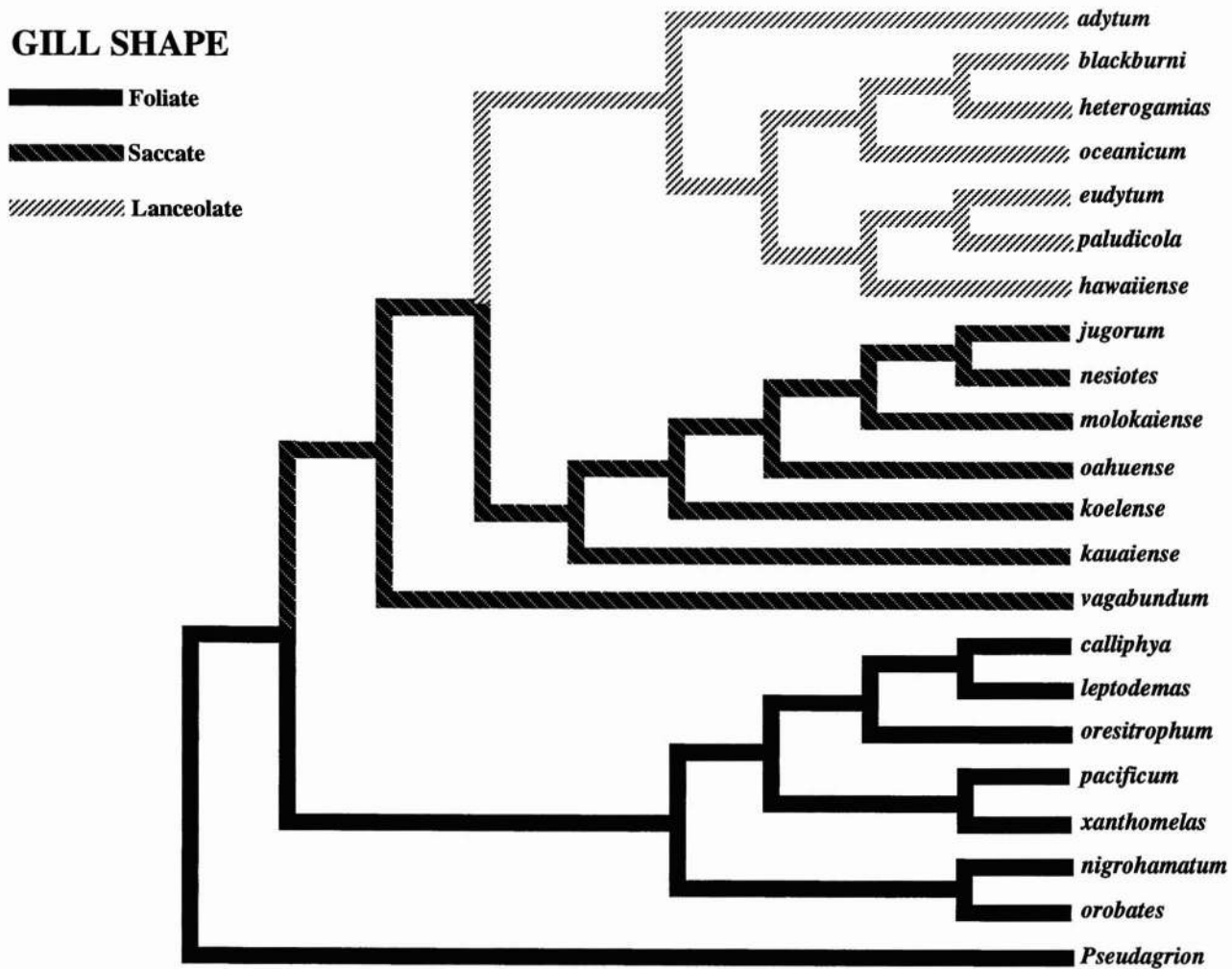


FIGURE 7. Gill types of *Megalagrion* species in relation to phylogeny. Note the obvious segregation of gill types among the major clades.

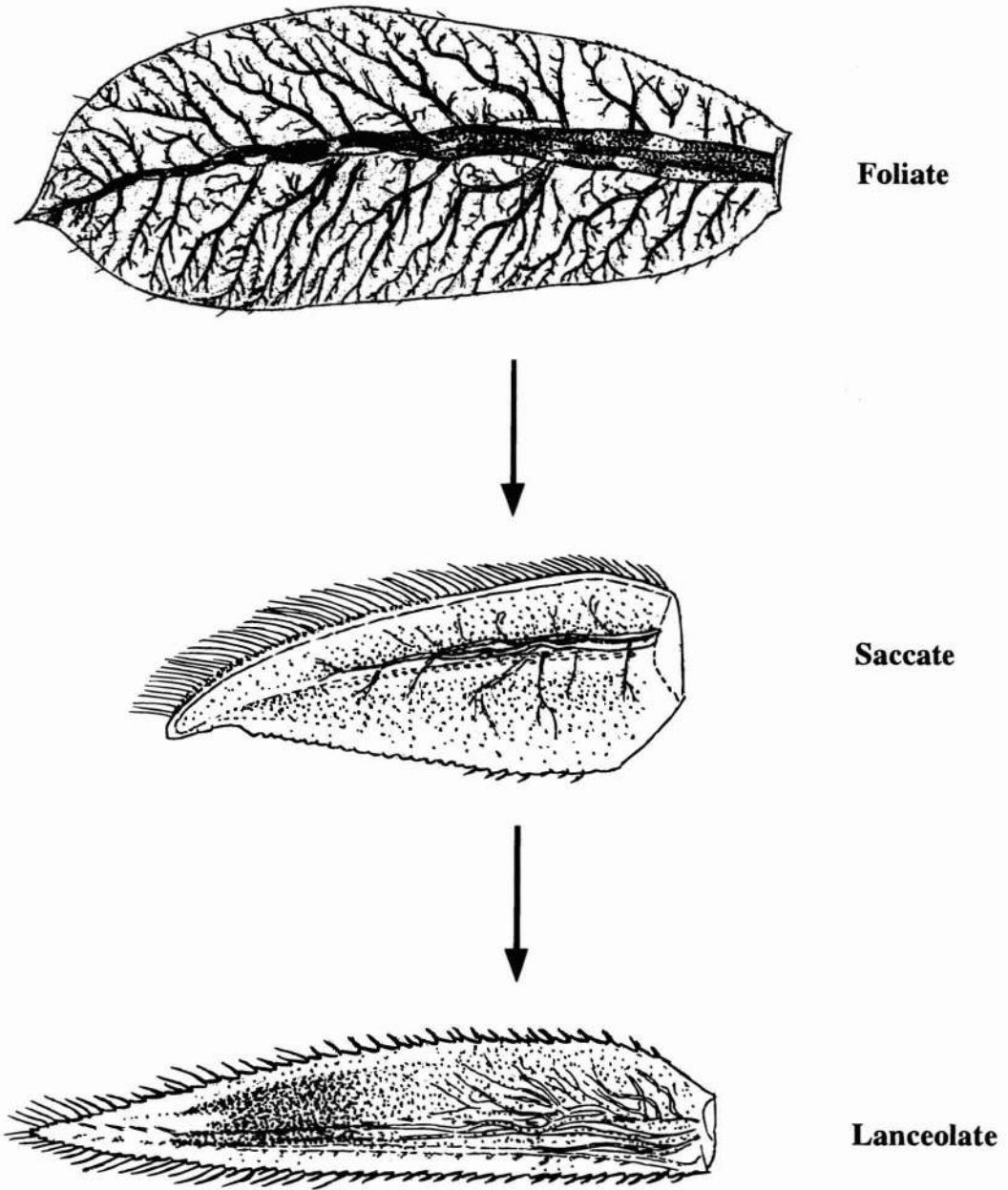


FIGURE 8. Hypothesized transformation series for gill shape among immatures of *Megalagrion* species based on phylogenetic analysis.

tence. A further prediction that may be derived from this analysis is that those species in the *oahuense* clade whose larvae are still unknown (*M. jugorum*, *M. molokaiense*, and *M. nesiotes*) will possess saccate gills.

CONSERVATION STATUS. Eight species or subspecies of *Megalagrion* are currently considered to be Threatened or Endangered as defined by the Endangered Species Act (U.S. Fish and Wildlife Service 1994), and another, *M. william-*

soni, is now believed to be extinct. The distribution of these taxa on the cladogram is shown in Figure 9 (with the exception of *M. williamsoni* [see Figures 1, 2]). In several cases these taxa are clustered within individual clades, but in other cases they are scattered as single taxa amid clades whose other species are not at risk. This indicates that two separate patterns of vulnerability are represented, one based on phylogenetically derived ecological vulnerability, the other on biogeographic misfortune.

In the *xanthomelas* clade, for instance, both of the included species, *M. xanthomelas* and *M. pacificum*, were formerly widespread throughout the Islands and bred in lowland habitats, which have been extensively altered by human use and heavily degraded by the introduction of exotic species. Similarly, the four terminal species in the *oahuense* clade, *M. williamsoni*, *M. molokaiense*, *M. jugorum*, and *M. nesiotis*, are hypothesized to be terrestrial breeders occupying specialized upland microhabitats. They are all single-island endemics that were apparently uncommon even when the first comprehensive collections of Odonata were made in Hawai'i by Perkins over 100 yr ago, and their inherent rarity and ecological specialization seem to have contributed to their decline in the face of extensive environmental perturbations. Both these groups of species can thus be viewed as possessing inherent phylogenetically based vulnerability due to their particular ecological preferences.

By contrast, the three remaining species at risk, *M. nigrohamatum nigrolineatum*, *M. leptodemus*, and *M. oceanicum*, are widely scattered across the cladogram and in all cases represent members of clades whose other members are not currently at risk. The single factor uniting all three species is their restriction to the island of O'ahu, the most heavily populated and ecologically disturbed of the main Hawaiian Islands. In this case it is not inherent phylogenetic vulnerability but rather unfortunate geographic circumscription that has led to their decline.

The rapid production and destruction of islands above the Hawaiian hot spot has led to a high rate of evolution and speciation among many elements of the Islands' biota, and *Mega-*

lagrion is an excellent example of this. All of the extant species are members of a monophyletic lineage that cannot have been present in the Islands for more than 25 million yr, based on current geological evidence (Carson and Clague 1995). The rapid diversification of forms evident in the current species assemblage is continuing apace, making the group a challenging problem in terms of delimiting taxa within the prevailing system of binomial scientific nomenclature. The current phylogenetic analysis now permits a first comprehensive glimpse of this diversification in an evolutionary context.

Based on the phylogenetic analysis presented herein, the following evolutionary scenario may be hypothesized: The initial ancestor of *Megalagrion* arrived from the Melanesian region, possessed larvae with foliate gills, and bred in standing or slow-moving waters. This ancestor reached Kaua'i or an even older island and underwent subsequent diversification into three major clades, one of which contained slow-water breeders that retained foliate gills, the other two of which contained seep breeders with saccate gills. One lineage of seep breeders retained the saccate gill type and subsequently produced species capable of breeding in phytotelmata; these phytotelmata breeders gave rise in turn to a set of terrestrially breeding species. The other set of seep breeders developed lanceolate gills and reached O'ahu, where they gave rise to a group of species specialized to breed in swift mid-stream waters, one member of which subsequently dispersed back to Kaua'i. These clades continued to disperse sequentially down the island chain as new islands were formed, giving rise to localized endemics on all Islands except the youngest island, Hawai'i.

With the arrival of humans in the Islands, these species were subjected to various degrees of novel perturbation, in the form of both habitat modification and contact with alien species. The initial Polynesian colonization probably had little effect on the upland species, but undoubtedly impacted the lowland taxa through the widespread conversion of terminal stream reaches to taro cultivation. With the arrival of European colonists the pace of change accelerated greatly. Within 100 yr the species breeding in lowland areas or terrestrial habitats were in danger of extinction on many Islands, and all stream-

CONSERVATION STATUS

- Not at risk
 Threatened
 Endangered

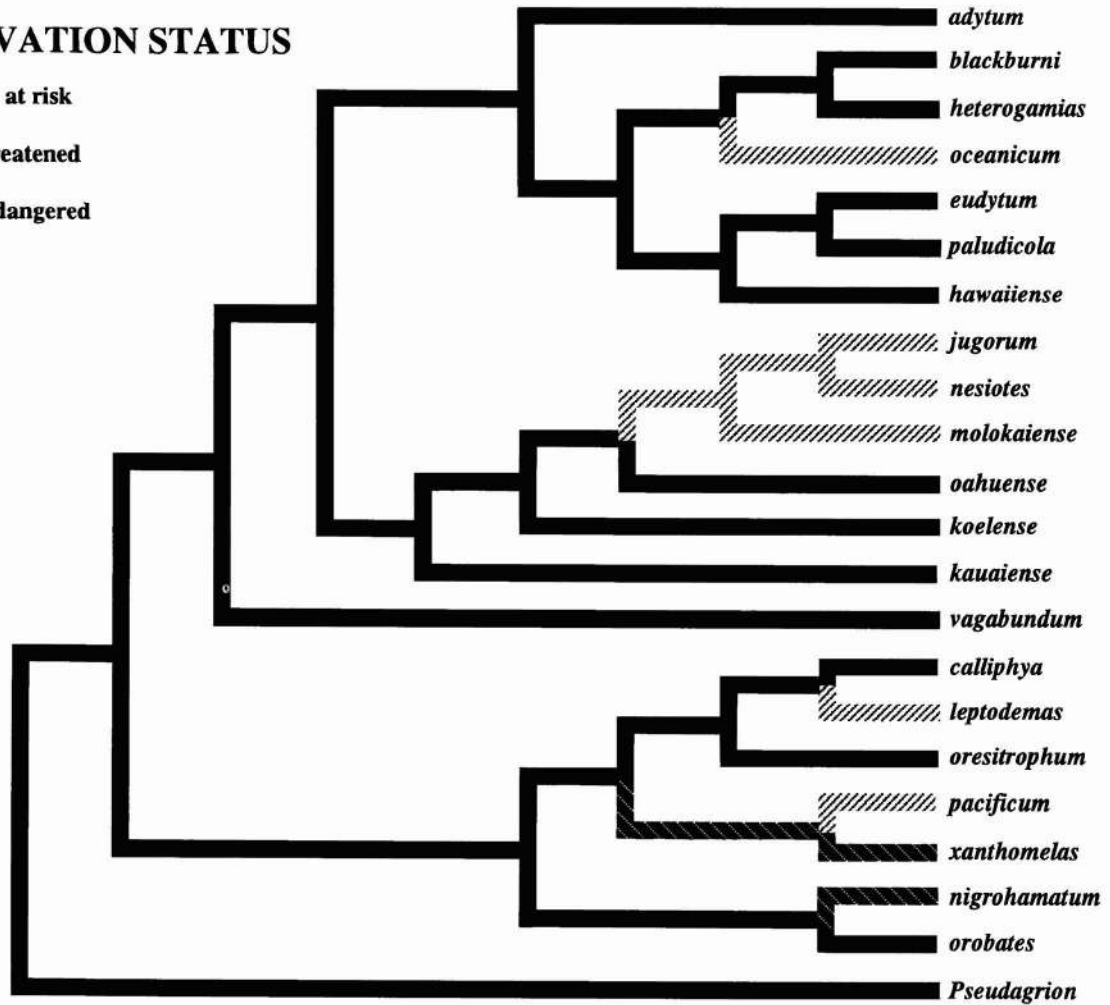


FIGURE 9. Conservation status of *Megalagrion* species in relation to phylogeny, with branches shaded to indicate status of individual species. Note that the species in the *xanthomelas* clade and the terminal section of the *oahuense* clade appear to be inherently vulnerable because of phylogenetic factors. The remaining taxa at risk, scattered across three separate clades, are those occurring on O'ahu and were compromised because of their geographic location.

breeding species on O'ahu were at risk. Despite recent conservation initiatives, it is still uncertain whether the extinction of certain particularly vulnerable species can be prevented.

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