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Phylogenetic Relationships of *Asclepias* (Apocynaceae) Inferred from Non-coding Chloroplast DNA Sequences

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Abstract—Milkweeds (*Asclepias* s. l., Apocynaceae) are characteristic perennial herbs of grasslands in North America and Africa that have long served as models for studying the evolutionary ecology of plant reproduction and plant defense. Generic circumscription of *Asclepias* has been long debated with recent workers favoring delimitation on geographic grounds; *Asclepias* s. s. is limited to the Americas and only segregate genera are recognized for African species. A widely used system introduced by Woodson classifies North American *Asclepias* into nine subgenera, with the largest subgenus, *Asclepias*, further divided into eight series. We investigated the phylogeny of *Asclepias* using three non-coding loci from the plastid genome: *rpl16* intron, *trnC*^{GCA}–*rpoB* spacer, and the adjacent *trnS*^{GCU}–*trnG*^{UUC} spacer and *trnG*^{UUC} intron. Parsimony, likelihood, and Bayesian analyses were conducted to evaluate hypotheses of continental and taxonomic monophyly. Hypothesis tests were conducted under the parsimony and likelihood criteria. We found moderate support for the monophyly of American *Asclepias* s. s. and for all but one representative of African *Asclepias* s. l. Within the Amerian clade, South American species are strongly supported as monophyletic and derived from North American ancestors. Only one of Woodson's 17 infrageneric taxa was found to be monophyletic. Monophyly of more than one half of the remaining 16 taxa could be statistically rejected using a conservative α level. Our results are consistent with taxonomic restriction of *Asclepias* to American species and single colonization events from Africa to North America and North America to South America. They also point to a need for major restructuring of infrageneric classification and future revsionary work.

Keywords—Africa, America, biogeography, classification, hypothesis test, milkweed.

Milkweeds (*Asclepias* L., Apocynaceae) are familiar plants to pollination biologists, chemical ecologists, lepidopterists, and increasingly to horticulturists. They have served as a preeminent model system for study of the evolutionary ecology of plant reproduction (Wyatt and Broyles 1994). Starting with the first experimental application of sexual selection theory to simultaneous hermaphrodites (Willson and Rathcke 1974; Willson and Price 1977), milkweeds have held center stage in the continuing debate over the significance of selection through male function in angiosperms (Broyles and Wyatt 1990, 1995; Fishbein and Venable 1996a; Broyles and Wyatt 1997; Queller 1997), and have played important roles in studies of plant hybridization (Kephart et al. 1988; Wyatt and Hunt 1991; Wyatt and Broyles 1992; Broyles et al. 1996; Broyles 2002), pollinator effectiveness (Willson and Bertin 1979; Willson et al. 1979; Morse and Fritz 1983; Fishbein and Venable 1996b), and size/number tradeoffs in plant reproduction (Fishbein and Venable 1996a). They have also served as an important model system for the study of plant/herbivore co-evolution, stemming from highly specialized interactions mediated by host-plant chemistry (Malcolm 1991; Farrell and Mitter 1998), particularly in the case of the well-known interaction with the Monarch butterfly (Brower et al. 1972; Malcolm and Brower 1989; Malcolm 1991).

Despite significant impacts made on the development of evolutionary theory by the study of individual milkweed species, only rarely have such studies been extended to the macroevolutionary level, due largely to a lack of understanding of the phylogenetic relationships among milkweed species. Farrell and Mitter (1998) reported co-speciation of *Asclepias* and a specialist herbivore, the cerambycid beetle, *Tetraopes*, but their analysis was based on an intuitive interpretation of a taxonomic classification (Woodson 1954b), not an explicitly phylogenetic hypothesis. Recently, Agrawal and Fishbein (Agrawal and Fishbein 2006, 2008; Agrawal et al. 2008, 2009a, b) have conducted phylogenetically explicit analyses of the evolution of defense traits in *Asclepias*, finding evidence for correlated evolution and evolutionary trends in these traits.

Asclepias is placed in the tribe Asclepiadeae Duby, characterized by pendulous pollinia (as opposed to horizontal or erect), and in the largely African subtribe Asclepiadinae Miq., characterized by an erect, usually herbaceous growth form (as opposed to twining, except for one species of *Pergularia* L.), and "hood-like" corona segments (Brown 1811; Decaisne 1844; Schumann 1895; Liede and Albers 1994). These coronal structures are exceptionally well developed in *Asclepias* and are highly diverse (Fig. 1). Diversification of coronas may be implicated in adaptation to differing suites of pollinators (cf. Grant 1952). However, rigorous testing of this hypothesis is hampered by the absence of accurate data on pollinator relationships for the great majority of species and a phylogenetic hypothesis for *Asclepias* on which to evaluate scenarios for the evolution of coronas (cf. Fishbein 2001).

There are approximately 130 species of *Asclepias* native to North America, including Mesoamerica and the Caribbean (Woodson 1954b; Blackwell 1964; McVaugh 1978; Holmgren and Holmgren 1979; Stevens 1983; Heil et al. 1989; Fishbein and Lynch 1999; Fishbein 2008; Fishbein et al. 2008). Approximately six additional species are native to South America (Bollwinkel 1969). A broad circumscription of *Asclepias* in North America

Fig. 1. Diversity of floral morphology in *Asclepias* and related genera, illustrated by representatives of major clades. A. *Pergularia daemia*, outgroup (cultivated, seed from Tanzania). B. *Calotropis procera*, outgroup (cultivated). C. *Gomphocarpus fruticosus*, clade C (cultivated, seed from Namibia). D. *Asclepias curassavica*, clade F (Querétaro, Mexico). E. *Asclepias subulata*, clade I (cultivated, seed from Sonora, Mexico). F. *Asclepias glaucescens*, clade J (Michoacán, Mexico). G. *Asclepias viridis*, clade K (cultivated, seed from Mississippi, U. S. A.). H. *Asclepias syriaca*, clade L (Virginia, U. S. A.). I. *Asclepias oenotheroides*, clade M (Oaxaca, Mexico). J. *Asclepias auriculata*, clade N (Oaxaca, Mexico). K. *Asclepias pedicellata*, clade O (Florida, U. S. A.). L. *Asclepias melantha*, clade P (Oaxaca, Mexico).

is well accepted and stems from the monographic work of Woodson (1941, 1954b), in which several well-known genera (e.g. *Acerates* Elliott, *Asclepiodora* A. Gray) were subsumed. The situation is complicated in Africa where up to 250 species have been or could potentially be included in *Asclepias*, depending on the breadth of circumscription and phylogenetic relationships among African and American species (Fishbein 1996; Goyder 2001b; see Table 1). The problem of the relationships among African and North American milkweeds has proved vexing to systematists specializing on the African species, who have swung from all-inclusive treatments under a broadly circumscribed *Asclepias* (Schumann 1895; Brown 1904, 1909) to exclusive treatments that recognize a dozen or more genera, resulting in the absence of any *Asclepias* s. s. native to the continent (Bullock 1952, 1953a, b, 1963; Nicholas and Goyder 1992; Goyder 1998a, b, 2001a, b; Goyder and Nicholas 2001). However, transfer of all African species to segregate genera is incomplete (Goyder 2001b), and a recent, pragmatic approach resubmerging several African genera into *Asclepias* has been taken, avowedly out of frustration with defining generic limits (Goyder 2009).

Resolution of the problem of the relationships among African and American species will bear on the understanding of the curious disjunction between these centers of diversity (Woodson 1954b; Fishbein 1996), an unusual biogeographic pattern that has been noted in other plant groups, for example *Bursera* Jacq. ex L./*Commiphora* Jacq. (Burseraceae), *Carpodiptera* Griseb. and *Hermannia* L. (Malvaceae), *Erblichia* Seem. (Turneraceae), *Nesaea* Comm. ex Kunth (Lythraceae), and the "annonoid" clade of Annonaceae (S. Graham 1977; Lavin and Luckow 1993; Doyle et al. 2004; A. Graham 2006; Weeks and Simpson 2007). More commonly, such centers of diversity in both Africa and North America are accompanied by significant diversity in biogeographically connecting regions, such as South America or Eurasia, but the absence of a fossil record for the genus in these regions suggests that they never have been important centers of diversity for *Asclepias*. The absence of paleobotanical evidence for *Asclepias*, however, is not compelling, as there is no rigorously verified fossil record for any milkweed (Apocynaceae subfam. Asclepiadoideae). There are three widely cited biogeographic scenarios that could generate African/North American disjunctions (Raven and Axelrod 1974): 1) Gondwanan vicariance followed by extinction in South America; 2) stepping-stone dispersal across the North Atlantic land bridge followed by extinction in Europe; and 3) stepping-stone dispersal across the Bering land bridge, followed by extinction in Asia. A less commonly invoked hypothesis is 4) long distance dispersal directly between Africa and North America.

A comprehensive classification of the North American species of *Asclepias* was formulated by Woodson (1941; 1954b), who recognized nine subgenera, with eight series within the largest, subg. *Asclepias* (Table 2; *A. fruticosa* L., which is adventive in the Americas, was included in a ninth series, Fruticosae but is placed now in *Gomphocarpus* R. Br.; see Goyder and Nicholas 2001). The names of the series, though not validly published by Woodson, will be used here for convenience. None of the infrageneric taxa were supported as monophyletic by the morphological phylogenetic analysis of Fishbein (1996); however, the strength of support for non-monophyly, as measured by the non-parametric bootstrap, was weak.

Current understanding of *Asclepias* systematics rests on the monographic studies of twentieth century systematists (e.g. Woodson 1954b), morphological phylogenetics (Fishbein 1996), and molecular systematic studies based on nuclear ribosomal ITS (S. P. Lynch, Louisiana State University-Shreveport, and L. E. Watson, Oklahoma State University, unpubl. data) and non-coding cpDNA sequences (Rapini et al. 2003, 2007; Goyder et al. 2007; Agrawal and Fishbein 2008). These phylogenetic studies have begun to clarify some of the broad scale patterns of relationships and now provide a solid framework for further investigation. However, these studies have been able to neither resolve much of the phylogeny of *Asclepias* nor provide strong support for putative clades.

Table 1. Overview and sampling of *Asclepias* s. l. outside of North America, after Goyder (2001b) for African species. All segregate genera are endemic to Africa. [a]endemic species only, excluding hybrids sensu Bollwinkel (1969) and *A. curassavica* and *A. woodsoniana*, which are also present in North America. [b]*Trachycalymma* was submerged in *Asclepias* by Goyder (2009).

Genus	Total Species (Sampled)	Reference
Asclepias (South America [a])	6 (5)	Bollwinkel (1969)
Gomphocarpus R. Br.	25 (3)	Goyder and Nicholas (2001)
Stathmostelma K. Schum.	13 (1)	Goyder (1998b)
Trachycalymma Bullock[b]	10 (2)	Goyder (2001a; 2009)
Glossostelma Schltr.	12 (1)	Goyder (1995)
Pachycarpus E. Mey.	37 (1)	Smith (1988); Goyder (1998a)
Aspidonepsis Nicholas & Goyder	5 (0)	Nicholas and Goyder (1992)
Aspidoglossum E. Mey.	34 (0)	Kupicha (1984)
Schizoglossum E. Mey.	12 (1)	Kupicha (1984)
Miraglossum Kupicha	7 (0)	Kupicha (1984)
Stenostelma Schltr.	3 (1)	Bullock (1952; 1956)
Parapodium E. Mey.	3 (0)	Brown (1909)
Woodia Schltr.	3 (0)	Brown (1909)
Periglossum Decne.	4 (0)	Brown (1909)
Cordylogyne E. Mey.	1 (0)	Brown (1909)
Fanninia Harv.	1 (0)	Brown (1909)
Margaretta Oliv.	1 (1)	Goyder (2005)
Xysmalobium R. Br. & residual *Asclepias* (Africa)	~80 (8)	cf. Goyder (2001b)
Undescribed or unplaced	~15 (0)	cf. Goyder (2001b)

Table 2. Classification of North American *Asclepias* following Woodson (1954b), the number of species in each infraspecific taxon, and sampling for this study. Woodson's series were not validly published.

Subgenus	Series	Species (+ subspp.)	Sampled
Asclepias	Incarnatae	16 (17)	15 (16)
	Tuberosae	3 (6)	3 (4)
	Exaltatae	9	9
	Grandiflorae	3	2
	Syriacae	11	11
	Purpurascentes	8	7
	Macrotides	8	6
	Roseae	13 (14)	13 (14)
Podostemma (Greene) Woodson		6	6
Anatherix (Nutt.) Woodson		1	1
Asclepiodella (Small) Woodson		7	7
Acerates (Elliott) Woodson		5	4
Solanoa (Greene) Woodson		3 (5)	3 (5)
Polyotus (Nutt.) Woodson		3	2
Asclepiodora (A. Gray) Woodson		7 (8)	6 (7)
Podostigma (Elliott) Woodson		4	4
Unclassified species described or resurrected since 1954		18	12
Total		**125 (132)**	**111 (117)**

Fishbein (1996) conducted the first phylogenetic analysis of *Asclepias*, which was based on a thorough sampling of North American species and a broad sampling of African species. Maximum parsimony (MP) trees found for this dataset were poorly resolved, but suggested that North American *Asclepias* may not be monophyletic. Although many of the African species fell outside the least inclusive clade containing all North American species, several were more closely related to North American than to other African species, but with negligible bootstrap support. The most intensive molecular phylogenetic analysis to date was conducted in the context of a phylogenetic analysis of evolutionary trends in defense traits, but did not address phylogenetic implications for *Asclepias* systematics (Agrawal and Fishbein 2008). Their Bayesian analysis of three non-coding cpDNA regions from 38 accessions was based on a subset of the data reported here. The present study expands sampling for the same cpDNA regions to a total of 151 accessions (127 representing the great majority of American species), and employs more extensive phylogenetic analyses, including parsimony and likelihood frameworks and hypothesis tests of alternative topologies.

The goals of the present study are to 1) assess the monophyly of the three continental areas of distribution for *Asclepias* (Africa, North America, South America), 2) explore hypotheses for the observed disjunctions among these three areas, 3) assess the monophyly of infrageneric taxa proposed by Woodson (1954b), and 4) resolve phylogenetic relationships that may form the basis for taxonomic revision of the genus.

Materials and Methods

***Taxon Sampling*—**Sampling includes 111 of approximately 125 species of North American *Asclepias* recognized by the authors (see Table 1). The total includes 107 species recognized by Woodson (1954a, b) and 18 species described since his monograph, rescued from synonymy (references cited in the introduction), or awaiting description. In addition, six of seven subspecific taxa recognized by Woodson (1954b, 1962) are included. Six species are represented by two or three samples to account for geographic variation and one sample of putatively hybrid origin is included. Thus, a total of 127 samples represent the North American species. Sampling also includes five of six species of *Asclepias* endemic to South America (Bollwinkel 1969). Of an estimated 250 species of *Asclepias* s. l. in Africa (Goyder 2001b), 19 are sampled, with one species represented by two subspecies. These accessions represent most of the segregate genera and other groupings discussed by Goyder (2001b). Together, these samples comprise an ingroup of 151 terminals representing approximately 143 taxa. Outgroups include the remaining genera of subtribe Asclepiadinae: one of two species of *Kanahia* R. Br. (Field et al. 1986), one of three species of *Calotropis* R. Br. (Rahman and Wilcock 1991), and one of two species of *Pergularia* L. (Fig. 1A; Goyder 2006). An additional outgroup, *Cynanchum ligulatum* (Benth.) Woodson from the related subtribe Cynanchinae is included to provide a firm root of the ingroup topology (Rapini et al. 2003; Liede-Schumann et al. 2005). A complete list of accessions, including voucher data and GenBank accession numbers, is included in Appendix 1.

***Character Sampling*—**Genomic DNA was extracted from silica-dried field collections or herbarium specimens using a modified CTAB protocol (Doyle and Dickson 1987) or a commercial kit (Wizard® Genomic DNA Purification Kit, Promega, Madison, Wisconsin). Genomic DNA served as templates for PCR amplification of three non-coding regions of the plastid genome: *rpl16* intron, *trnC*^{GCA} *–rpoB* intergenic spacer, and the contiguous *trnS*^{GCU}*–trnG*^{UUC} intergenic spacer/*trnG*^{UUC} intron, regions that typically exhibit among the highest per-site substitution rates in the plastid genome (Jordan et al. 1996; Small et al. 1998; Ohsako and Ohnishi 2000; Kelchner 2002; Shaw et al. 2005). The PCR reactions were carried out with the DNA Engine™ PTC-200 (MJ Research, Waltham, Massachusetts) and the iCycler® (Bio-Rad Laboratories, Hercules, California). Although recommended cycling parameters were tested for each region, the great majority of DNA sequencing templates were generated with a low-stringency protocol developed for the *rpl16* intron (Small et al. 1998). This protocol consists of an initial temperature of 80°C for 4 min, followed by 35 cycles of 1) 94°C for 1 min, 2) 50°C for 1 min, and 3) ramp to 65°C at 0.3°C/min-65°C for 5 min, followed by a final extension at 65°C for 5 min and a hold at 4°C. A standard recipe for 50 µl reactions consisted of 1 µl of genomic DNA undiluted or diluted by a factor of 10–100, 0.5 mM amplification primers, 200 µM dNTPs, 1.5 mM $MgCl_2$, 1 × reaction buffer supplied by the polymerase manufacturer, 5% DMSO, and 0.2–0.5 U *Taq* DNA polymerase (Promega). Difficult templates were amplified by substituting HotMaster® *Taq* DNA polymerase (Eppendorf, Westbury, New York) and omitting DMSO.

DNA of each region was amplified using universal primers: rpl16F71 and rpl16R1661 (Jordan et al. 1996) for the *rpl16* intron, trnC5′R and rpoB5′R (Ohsako and Ohnishi 2000) for the *trnC*^{GCA}*–rpoB* spacer, and trnS^{GCU} and 3′trnG^{UUC} (Shaw et al. 2005) for the *trnS*^{GCU}*–trnG*^{UUC} spacer/*trnG*^{UUC} intron. Difficult genomic templates, typically obtained from older herbarium specimens, required amplification of smaller fragments by pairing each universal primer with an "internal" primer specific to *Asclepias* s. l. (see below) in separate reactions. Amplicons were prepared for DNA sequencing by one of several methods: ethanol/acetate precipitation, ExoSAP-IT degradation of oligonucleotides, or column filtration (Wizard® SVGel and PCR Cleanup System, Promega, or QiaQuick PCR Purification Kit, Qiagen, Valencia, California).

DNA sequences were obtained by direct cycle sequencing with ABI Prism® BigDye Terminator™ cycle sequencing ready reaction kit (Perkin-Elmer Biosciences, Waltham, Massachusetts), BigDye® terminator v3.1 cycle sequencing kit (Applied Biosystems, Foster City, California), and CEQ™ dye terminator cycle sequencing kit (Beckman-Coulter, Fullerton, California), following the manufacturers' protocols, but with reaction mixtures diluted by a factor of 0.5 or 0.25. Dye-labeled fragments were analyzed on the ABI Prism® 377 DNA Sequencer (Perkin-Elmer Biosciences) in the Department of Biology, University of Idaho, the ABI Prism® 3100 Genetic Analyzer at the Oregon Health Sciences University Sequencing Core (Applied Biosystems), or the CEQ™ 8000 Genetic Analysis System (Beckman-Coulter) at the Mississippi State University Life Sciences Biotechnology Institute. Sequencing primers gave complete, double stranded coverage and included both universal amplification primers (see above) and *Asclepias*-specific primers (Agrawal and Fishbein 2008). Complete sequences were assembled and edited with Sequencher™ ver. 3.0 (Gene Codes Corp., Ann Arbor, Michigan and the SeqMan™II module of Lasergene ver. 6 (DNASTAR, Madison, Wisconsin).

***Phylogenetic Analyses*—**Sequences were aligned by eye independently for each of three regions with the aid of Se-Al ver. 2.0 (Rambaut 1996) and MacClade ver. 4.08 (Maddison and Maddison 2005). Alignments are available on TreeBASE (study number S11225). Many of the required gaps were easily interpreted as independent insertion/deletion (indel) events. However, each of the three regions contained at least one stretch of overlapping gaps that could be aligned only with substantial ambiguity; such regions were excluded from further analysis.

Analytical approaches included tree searches under the maximum parsimony (MP) and maximum likelihood (ML) criteria and estimation of the distribution of posterior probabilities of tree topologies under the framework of Bayesian inference. All analyses were conducted on a Power Mac Quad 2.5 GHz personal computer with 8 GB RAM. The three loci were analyzed simultaneously because of complete linkage in the plastome, ensuring that the sequences have identical phylogenetic histories. Variation in mutational processes across the regions was explored in partitioned Bayesian analyses. Separate parsimony analyses treated gaps as missing data or recoded as multistate indel characters following the reasoning of Lutzoni et al. (2000); as gap treatment minimally affected phylogenetic results, only indel-coded results are discussed further because indel characters increased bootstrap support for some nodes.

Parsimony analyses used a two-tiered approach of ratchet analysis (Nixon 1999), implemented with PAUPRat ver. 1 (Sikes and Lewis 2001) and PAUP* ver. 4.0b10 (Swofford 2002), followed by tree-bisection-reconnection (TBR) branch swapping. Ratchet analysis employed an optimal 25% re-weighted characters; 20 independent analyses were conducted with 200 rounds of re-weighting each and TBR branch swapping limited to a single MPT. Ratchet MPTs were summarized by a strict consensus tree (Nixon 1999) and also used as starting trees for further TBR branch-swapping, with the number of saved trees capped at 2×10^5 by the limitation of computer memory.

Clade support for parsimony trees was measured using non-parametric bootstrapping (BS; Felsenstein 1985) and decay index/Bremer support (DI; Donoghue et al. 1992; Bremer 1994). Bootstrapping was implemented

in PAUP* with 5,000 pseudoreplicates and two random addition starting trees subjected to TBR branch-swapping, with 10 trees retained for each pseudoreplicate; caution should be used in interpreting the results because search parameters were not identical to those used for finding MPTs. Decay indices were calculated using TreeRot, version 3 (Sorenson and Franzosa 2007), with 20 random addition starting trees and 100 trees retained. Results were inspected to verify that the minimal tree length was obatined in calculating the decay indices.

Maximum likelihood trees were sought using the likelihood ratchet (Morrison 2007) implemented in PRAP2.0b3 (Müller 2004). Ten iterations of the ratchet were performed along with subtree pruning-regrafting (SPR) branch swapping. Choice of nucleotide substitution model was made using hierarchical likelihood ratio tests (hLRTs) and the Akaike information criterion (AIC) as implemented in MrModeltest, version 2 (Nylander 2004). Maximum likelihood estimates of model parameters were made on a neighbor joining tree produced in PAUP*.

Bayesian inference was conducted using MrBayes, version 3.1.2 (Ronquist and Huelsenbeck 2003) to explore model heterogeneity across partitions and evaluate support for clades present in the ML tree. The optimal evolutionary model of nucleotide substitution was chosen for each data partition (cpDNA locus) by applying hLRTs and the AIC, as implemented in MrModeltest, version 2 (Nylander 2004). Partitioned Bayesian analyses employed independent evolutionary models for the three loci (Suchard et al. 2003; Nylander et al. 2004). Metropolis-coupled Markov chain Monte Carlo (MCMCMC) simulations were run with eight linked chains (seven heated and one cold) and default priors for model parameters (all uninformative), except as follows. Preliminary analyses were run to optimize the parameters of the MCMCMC simulations (Ronquist and Huelsenbeck 2003) resulting in an increase in the Dirichlet parameter for the cross-partition rate multiplier to 50,000 and the reduction of the parameter controlling the temperatures of heated chains to 0.02. Two independent runs of 5×10^6 generations were compared to assess convergence to a stationary distribution of parameter samples by examining the average of standard deviations of split frequencies between the two runs in MrBayes and calculating the effective sample size of parameter values visited by the Markov chains using Tracer, version 1.4 (Rambaut and Drummond 2007). A cut-off of 0.015 standard deviations and an effective sample size of 100 were used as guidelines to assess convergence of runs. Parameter values and trees sampled every 100 generations from the stationary distribution were used to calculate posterior probabilities.

***Hypothesis Tests*—**Tests were conducted for the significance of alternative phylogenetic hypotheses under parsimony and likelihood frameworks. We employed Templeton's implementation of the Wilcoxon signed-ranks test (Templeton 1983), the winning sites test (Prager and Wilson 1988), and the parsimony implementation of the Kishino-Hasegawa (KH) test (Kishino and Hasegawa 1989) under parsimony, and the likelihood-based Shimodaira-Hasegawa (SH) test (Shimodaira and Hasegawa 1999), all implemented in PAUP*. Optimal trees under parsimony and likelihood were compared to optimal trees under constraints representing the following a priori hypotheses: 1) North American *Asclepias* is monophyletic, 2) South American *Asclepias* is monophyletic, 3) American *Asclepias* is monophyletic, 4) African *Asclepias* is monophyletic, and 5) each infrageneric taxon proposed by Woodson (1954b) is monophyletic. For any hypothesis that was congruent with the optimal parsimony and likelihood trees, the converse of the hypothesis was tested (e.g. American *Asclepias* is NOT monophyletic). To maintain table-wide significance of $p < 0.05$, sequential Bonferroni correction was employed (Rice 1989). Hypotheses 1–4 bear on the biogeographic history of the Americas. Monophyly of each geographic region is more consistent with vicariant processes (e.g. Gondwanan breakup) or extremely rare dispersal events than a history of repeated dispersal events. Conversely, polyphyly is more consistent with a history influenced more by dispersal. An intermediate scenario of monophyletic biogeographic areas nested in paraphyletic areas could result from recent dispersal or vicariant events (e.g. migration over Beringian or North Atlantic land bridges).

Results

***Sequence Data*—**Nucleotide sequences were aligned easily by eye with the exception of regions containing direct repeats, particularly mononucleotide repeats. Such regions were excluded from further analysis. The three regions were comparable in terms of aligned length, proportion of potentially informative sites and number of gaps that could be unambiguously coded as insertion/deletion characters (Table 3). There was a low percentage of missing data due mostly to incomplete sequences, although one sample could not be sequenced for the *rpl16* intron (*Margaretta rosea* Oliv.) and two could not be obtained from the *trnS–trnG–trnG* region [the Kansas accession of *Asclepias tuberosa* L. spp. *interior* Woodson, *A. pringlei* (Greenm.) Woodson].

***Phylogeny of Asclepias*—**Hierarchical likelihood ratio tests differed in the selected model depending on which decision path was employed. For the *rpl16* intron, either the F81 + I + Γ or GTR + I + Γ model was chosen; the AIC selected GTR + I + Γ, which was strongly favored over F81 + I + Γ (deltaAIC = 85). For the *trnC–rpoB* spacer, either the F81 + Γ or GTR + Γ model was chosen by hLRTs; the AIC selected GTR + Γ, which was strongly favored over F81 + Γ (deltaAIC = 156). For the *trnG–trnS* spacer/*trnG* intron, the GTR + Γ model was chosen by all hLRTs and the AIC. For maximum likelihood analysis, the GTR + I + Γ was selected as the single model that best fit all three combined loci. For Bayesian analysis, this model was applied to all three regions, with parameter estimates unlinked across the regions. Maximum likelihood estimates (MLEs) of model parameters for the combined data set fell within the range of the median values obtained in the stationary distributions of the Bayesian analysis for the three gene regions (Table 4). Parameter estimates were similar across partitions, except that the rates of some substitution types varied more between the *trnS–trnG–trnG* region and the other two regions and that the estimate of α (shape parameter of the gamma distribution of among-site rate variation) for the *rpl16* intron region was much lower than that of the other two regions.

Phylogenetic estimates under parsimony and likelihood were largely congruent, with slightly more resolution in the maximum likelihood tree than the strict consensus of MPTs. The same ML tree (Fig. 2) was discovered in each of the 10 ratchet iterations (lnL = -12,577.17728). Extreme branch length heterogeneity is apparent; within each major clade there is a combination of "comblike" structure suggestive of rapid diversification and at least one subclade containing long branches (e.g. clade F in Fig. 2A, clade J in Fig. 2B, and the terminal branch of *Asclepias* sp. nov. cf. *notha* W. D. Stevens in Fig. 2C). Each of the 20 ratchet analyses resulted in the discovery of MPTs of length 1,127. Further TBR branch

Table 3. Attributes of the aligned sequences of three plastid loci (*rpl16* intron, *trnC–rpoB* intergenic spacer, *trnS–trnG* intergenic spacer/*trnG* intron) sequenced for 155 accessions of *Asclepias* s. l. and outgroups.

Locus	Aligned Length (bp)	Length After Ambiguously Aligned Regions Excluded (bp)	Variable Sites Included (bp)	Parsimony Informative Sites Included (bp)	Missing Data	Indels Coded as Multistate Characters
rpl16	1,805	1,112	128	89 (8.0%)	0.7%	11
trnC–rpoB	1,614	1,132	156	99 (8.7%)	0.3%	11
trnS–trnG–trnG	1,447	1,168	138	103 (8.8%)	1.4%	11
Total	4,866	3,412	422	291 (8.5%)	0.8%	33

TABLE 4. Parameter estimates (median) of the GTR + I + Γ substitution model sampled during Bayesian analysis with parameters unlinked across three data partitions. Maximum likelihood estimates (MLEs) for the combined data set are presented for comparison. r = reversible substitution rate between indicated bases; π = stationary frequency of indicated base; p_{inv} = proportion of invariable sites; α = parameter of gamma distribution fit to rate variation among sites free to vary.

	Parameter										
Partition	r_{AC}	r_{AG}	r_{AT}	r_{CG}	r_{CT}	π_A	π_C	π_G	π_T	p_{inv}	α
rpl16 intron	0.967	0.764	0.270	0.226	0.806	0.39	0.16	0.18	0.27	0.55	0.05
trnC–rpoB	0.746	0.653	0.108	0.304	0.591	0.35	0.16	0.13	0.35	0.26	1.25
trnS–trnG–trnG	1.47	1.15	0.138	0.212	1.40	0.34	0.15	0.18	0.33	0.44	1.56
combined (MLE)	0.991	0.845	0.153	0.246	0.868	0.36	0.15	0.16	0.32	0.39	1.05

swapping in PAUP* did not discover any trees incompatible with the strict consensus of the trees found in the ratchet analysis (not shown).

The ML tree and strict consensus of MPTs show a great deal of structure at the base of *Asclepias* s. l., and in numerous nested clades, but basal relationships in a clade of wholly American species are largely unresolved (clade B in Fig. 2A, with subclades D and E expanded in Figs. 2B, C). *Asclepias* s. l. (clade A in Fig. 2A) is well supported as monophyletic within Asclepiadinae (PP 1, BS 100, DI 16). Within this clade, 19 African accessions form a clade (C; see Fig. 1C) with support that varies depending on metric (PP 1, BS < 50, DI 1) and all 124 American accessions form a clade (B), also with varying measures of support (PP 1, BS < 50, DI 1). These two major clades together form a clade with variable support (PP 0.97, BS < 50, DI 1), to the exclusion of African *Trachycalymma pseudofimbriatum* Goyder. The exclusive placement of *T. pseudofimbriatum* was unexpected, and the DNA sequences were verified by re-sequencing newly obtained material, kindly provided by D. Goyder from the same collection (the type). Within the African clade, relationships among species are poorly resolved. The two sampled species of *Xysmalobium* R. Br. are strongly supported as a clade (PP 1, BS 97, DI 5). There was some support for the non-monophyly of *Gomphocarpus*, with *G. cancellatus* (Burm. f.) Bruyns placed as sister to *Asclepias aurea* (Schltr.) Schltr. (PP 0.90). African species that have not been reassigned from *Asclepias* to other genera do not themselves form a clade.

Within the wholly American clade (B in Fig. 2A) most species fall into one of three subclades: a clade (D, expanded in Fig. 2B) containing 58 accessions of species with mostly more northern, temperate distributions (PP 0.95, BS < 50, DI 1), a more strongly supported clade (E, expanded in Fig. 2C) containing 31 accessions of species distributed mostly in montane regions of Mexico (PP 1, BS 77, DI 2), and a strongly supported clade (F; see Fig. 1D) containing all but one of the sampled species of Woodson's *Asclepias* series Incarnatae plus all five sampled species of South American *Asclepias* (PP 1, BS 100, DI 8). The South American species form a well-supported clade (G; PP 1, BS 99, DI 4) that in turn is nested within a well-supported clade (unlabeled; PP 1, BS 100, DI 8) along with a pair of ser. Incarnatae species, *A. curassavica* L. (Fig. 1D) and *A. nivea* L. All remaining species of ser. Incarnatae, except *A. leptopus* I. M. Johnst., which is placed as the sister to clade I, form a well-supported clade (H; PP 1, BS 100, DI 9). The sister group of the emended ser. Incarnatae (clade F) consists of Californian species, *A. californica* and *A. vestita*, but is only supported in the ML analysis (PP 0.86). Remaining species of clade B are unresolved or are placed in weakly to strongly supported clades, notably clade I of nearly leafless shrubs native to the Sonoran Desert (PP 1, BS 99, DI 4; see Fig. 1E). The successive sister taxa of clade I are *A. leptopus* (PP 1, BS 77, DI 1) and *A. cutleri* Woodson (PP 0.97, BS 68, DI 1).

Relationships are poorly resolved within the large clade (D) of American species distributed mostly north of the U. S. A.-Mexico border (Fig. 2B). Within this clade are several subclades of more than three species with varying levels of support. One small subclade (J) of atypical distribution for clade D is well supported (PP 1, BS 100, DI 14) and contains six glaucous-leaved species limited almost entirely to Mexico and Central America (see Fig. 1F). A second subclade (K) that is well supported (PP 1, BS 91, DI 3) contains species with distributions in the southwestern U. S. A. and northern Mexico (see Fig. 1G). A third subclade (L) contains species distributed primarily in the eastern U. S. A. and Canada (PP 0.99, BS 76, DI 2). This clade contains some of the more familiar milkweeds of the U. S. A., such as *A. syriaca* L. (see Fig. 1H) and *A. tuberosa* L. The sister to this group, *A. speciosa*, is supported by Bayesian analysis (PP 0.91, BS < 50, DI 1). Most species in a fourth subclade (M) have distributions that straddle the U. S. A.-Mexico border, with ranges of some extending to Central America or the U. S. A. Midwest (PP 1, BS 82, DI 2; see Fig. 1I). This clade contains *A. arenaria* Torr. and all sampled members of subgenus *Podostemma*, except *A. subulata* Decne. (see Fig. 1E). There is Bayesian support for a clade (O) of three species endemic to pine flatwoods in Florida and adjacent states (PP 0.95; see Fig. 1K).

Relationships in the clade of highland Mexican species are not well resolved (clade E in Fig. 2A, expanded in Fig. 2C). About one half of the species are placed in a weakly supported clade (N; PP 0.92, BS 54, DI 1; see Fig. 1J). Remaining species are placed in a second clade (P; see Fig. 1L) only in the ML tree (PP 0.82). Some structure is apparent in these two clades, with groups of two to four species well supported.

Species monophyly was not supported in all cases in which sampling permitted such evaluation. Strong support for monophyly was found for *Gomphocarpus fruticosus* (L.) W. T. Aiton (PP 1, BS 100, DI 8; Figs. 1C, 2A), *Asclepias cryptoceras* S. Watson (PP 1, BS 100, DI 7; Fig. 2A), *A. speciosa* Torr. (PP 1, BS 99, DI 7; Fig. 2B), and *A. scheryi* Woodson (PP 1, BS 99, DI 4; Fig. 2C). There was variable support for the monophyly of *A. incarnata* L. (PP 1, BS 65, DI 1; Fig. 2A) and *A. asperula* (Decne.) Woodson (PP 0.99, BS 64, DI 1; Fig. 2B). There was no support for or against the monophyly of *A. similis* Hemsl. (Fig. 2C) and extremely weak support for the non-monophyly of *A. subverticillata* (A. Gray) Vail (PP 0.51; Fig. 2A). There was moderate to strong support for the non-monophyly of *A. tuberosa*, with *A. obovata* Elliott placed as sister to *A. t.* subsp. *interior* Woodson, to the exclusion of *A. t.* subsp. *rolfsii*

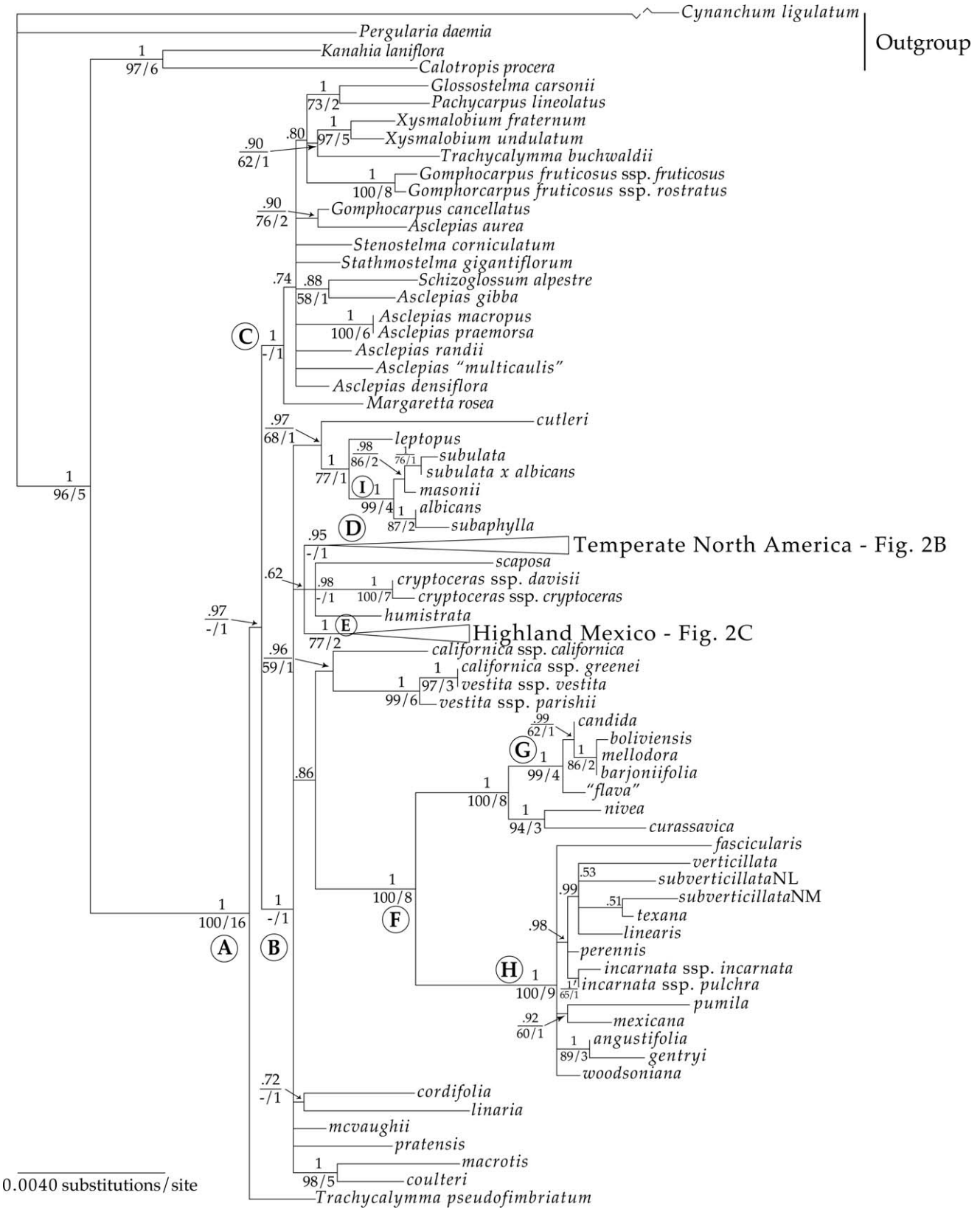

FIG. 2A. Maximum likelihood phylogram discovered in 10 independent ratchet analyses. Note the broken branch to the outgroup, *Cynanchum ligulatum*, shortened to facilitate presentation. With the exception of a few weakly supported nodes, this topology is identical to the strict consensus of most parsimonious trees discovered in 20 independent ratchet analyses. Taxa lacking generic names are all American species of *Asclepias*. Clade support is indicated near each node with Bayesian posterior probabilities above non-parametric bootstrap percentages and decay indices (PP over BS/DI). Nodes lacking both bootstrap percentages and decay indices are not present in the strict consensus of MPTs. Circled letters indicate clades discussed in the text. The specific epithets "flava" and "multicaulis" are in quotes because the names are illegitimate and no valid alternatives are available. Multiple accessions of the same species are distinguished by state of collection as follows: CA, California, U. S. A.; JAL, Jalisco, Mexico; KS, Kansas, U. S. A.; MICH, Michoacán, Mexico; MS, Mississippi, U. S. A.; NL, Nuevo Leon, Mexico; NM, New Mexico, U. S. A.; QRO, Querétaro, Mexico.

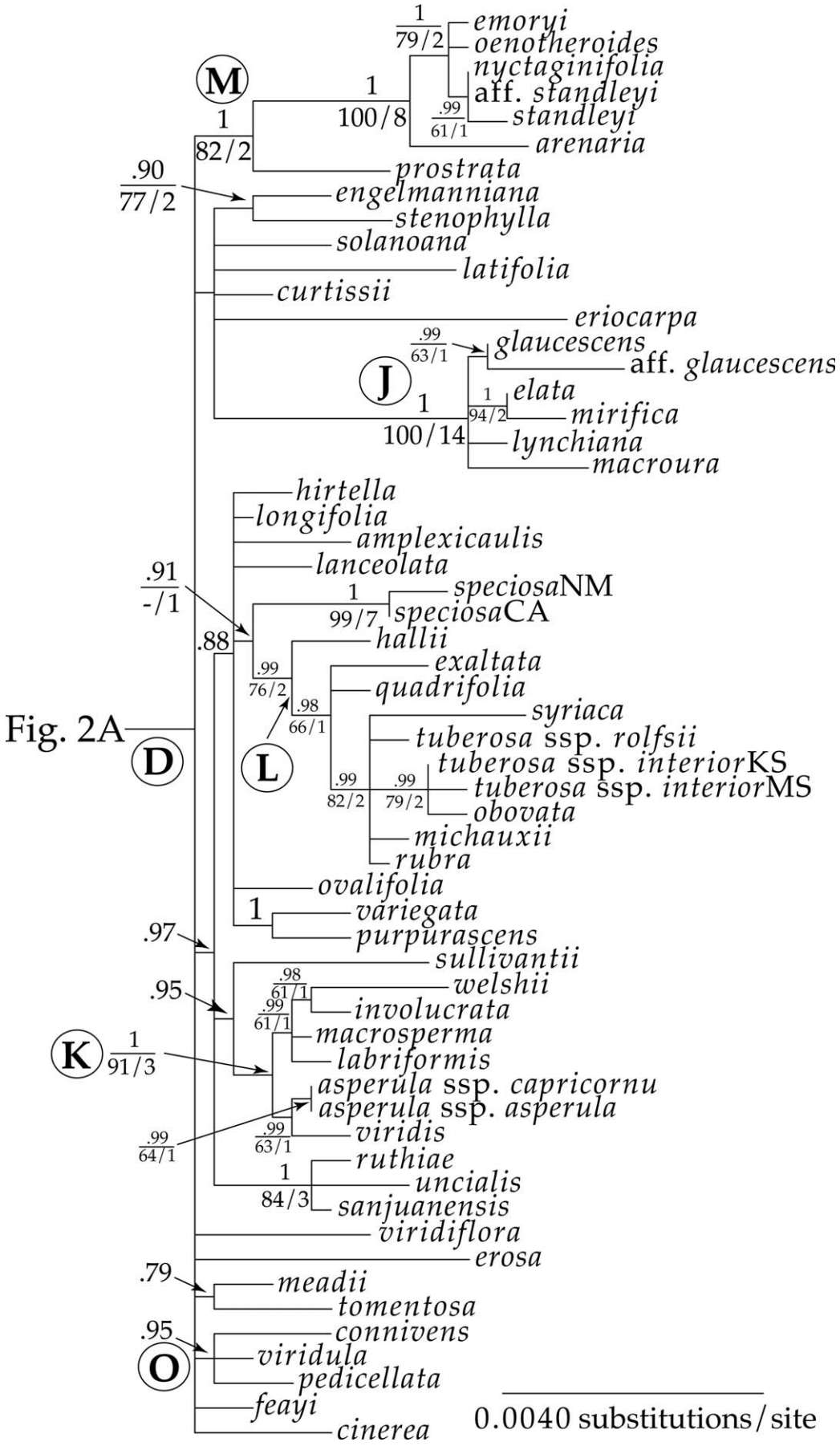

FIG. 2B. Figure 2 continued. Temperate North American clade (clade D in Fig. 2A).

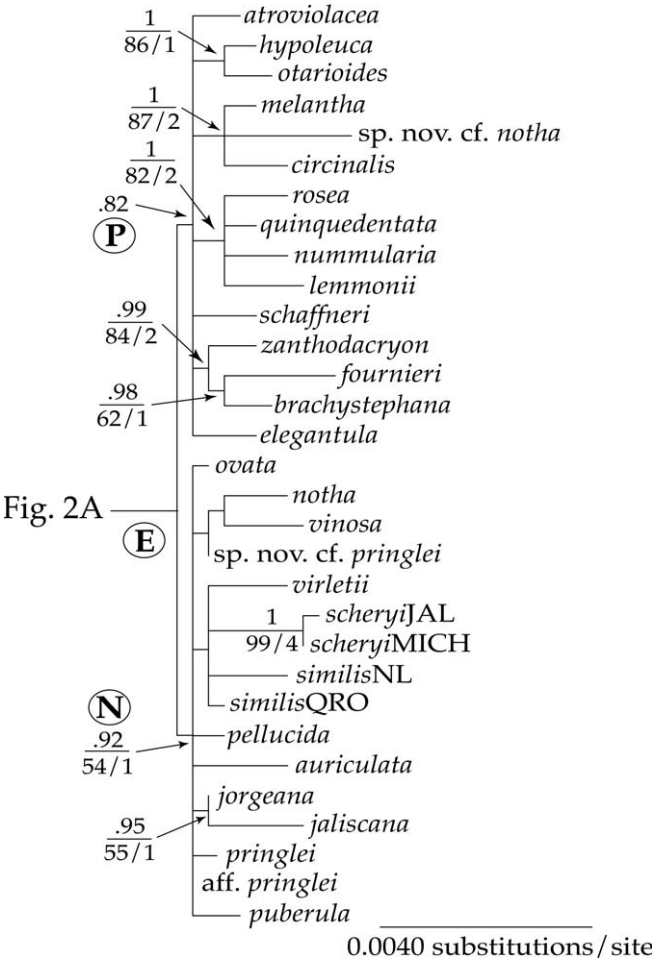

FIG. 2C. Figure 2 continued. Highland Mexican clade (clade E in Fig. 2A).

(Britton ex Vail) Woodson (PP 0.99, BS 79, DI 2; Fig. 2B). There was strong support for the non-monophyly of *A. californica* Greene and the paraphyly of *A. vestita* Hook. & Arn., with *A. c.* subsp. *greenei* Woodson placed as sister to *A. v.* subsp. *vestita* (PP 1, BS 97, DI 3; Fig. 1A).

***Hypothesis Tests*—**Tests of group membership in a parsimony framework generally gave the same result, regardless of the method employed (i.e. winning sites, Templeton, KH tests) and these corresponded well with the SH tests conducted under the likelihood framework (Table 5). Each of Woodson's (1954b) infrageneric taxa was tested (i.e. eight subgenera and eight series of subg. *Asclepias*), except the monospecific subg. *Anatherix*. Only one of these 17 taxa, subgenus *Polyotus* with two of three species sampled, was monophyletic in the strict consensus of MPTs or in the ML tree. Optimal trees (MP or ML) lacking a monophyletic *Polyotus* were not significantly less optimal than unconstrained trees, indicating that the non-monophyly of this subgenus cannot be rejected (Table 5). All other infrageneric taxa were not monophyletic in the unconstrained MPTs and ML tree, and tests were conducted to determine whether trees in which each taxon was constrained to monophyly were significantly less optimal. Monophyly of more than half of the taxa was rejected following sequential Bonferroni correction by two or three of the parsimony tests and by the SH test: subg. *Asclepias*, subg. *Podostemma*, subg. *Solanoa*, subg. *Asclepiodora*, ser. Incarnatae, ser. Exaltatae, ser. Syriacae, ser. Macrotides, and ser. Roseae (Table 5). Probabilities for several other tests were low, but not significant following correction. Parsimony and likelihood trees supported an emended ser. Incarnatae, in which *A. leptopus* is excluded and all South American species included. However, trees in which this emended Incarnatae is constrained to be non-monophyletic are not significantly less optimal following probability correction (Table 5).

In optimal parsimony and likelihood trees, geographic clades are formed by all American species and by all South American species, whereas all African species do not form a clade, nor do all North American species. Trees in which either American species or South American species are constrained to be non-monophyletic are not significantly less optimal (Table 5). Trees in which African species are constrained to be monophyletic also are not significantly less optimal. However, trees in which North American species are constrained to be monophyletic have low p values, although these are significant only for the parsimony KH test following correction (Table 5).

DISCUSSION

Asclepias s. l. is a species-rich clade of charismatic plants characteristic of the grasslands of North America and Africa. With centers of diversity on both continents and poor representation in South America, several scenarios for the origin and diversification of this clade can be hypothesized. Our results support a scenario in which *Asclepias* s. l. originated in Africa, migrated only once to North America, and thence only once to South America, although statistical significance for some key nodes bearing on this scenario was not attained with our current data and strict hypothesis-testing approach. All other genera of Asclepiadinae occur in Africa or both Africa and Asia, suggesting an African origin of *Asclepias* s. l. The times of origin of *Asclepias* s. l. and the wholly American *Asclepias* s. s. are difficult to ascertain in the absence of fossil calibrations for molecular dating. However, the low levels of sequence divergence among species, the nesting of *Asclepias* high within the Apocynaceae (Potgieter and Albert 2001), and the derivation of South American species from North American ancestors would seem to argue against Woodson's (1954b) preferred hypothesis of a Gondwanan disjunction in *Asclepias.* Futhermore, monophyly of all American and nearly all African species argues against frequent dispersal between these centers of diversity. Our results are consistent with Africa to America migration via either the North Atlantic or Bering land bridges or via long-distance transoceanic dispersal. Until recently, the possibility of range expansion of temperate or subtropical taxa through the North Atlantic route was thought to have ended in the early Eocene (Tiffney 1985). This timing (40–50 my before present) could be too early for the migration of *Asclepias* to the Americas. However, recent paleobotanical discoveries and dated phylogenies for several groups have increasingly supported the hypothesis that range expansion across the North Atlantic may have been possible until the mid-Miocene or even later (Tiffney 2008). Because recent long-distance dispersal could have occurred at any time, it must be considered as an alternative hypothesis, especially if the time of origin of the American-African disjunction is found to be too recent for migration over Tertiary land bridges. A node-dated phylogeny for this clade will aid in refuting alternative biogeographic hypotheses.

TABLE 5. Hypothesis tests for selected a priori clades based on Woodson's infrageneric taxa and geography. In addition, a post hoc test for an emended series Incarnatae (i.e. omitting *A. leptopus* and including all South American species) found in all phylogenetic analyses is included. When clades are present in optimal phylogenetic trees (MP and ML), the probabilities of the best trees lacking those clades are reported. When clades are not present in optimal phylogenetic trees, the probabilities of the best trees bearing those clades are reported. Significant *p* values following sequential Bonferroni correction indicated by asterisk (*).

Putative Clade	Present in MPTs and ML Tree?	Length Difference (steps)	Parsimony KH test *p* value	Templeton test *p* value	Winning sites test *p* value	Likelihood Difference	SH test *p* value
Tests of Taxonomic Clades							
Subgenus *Asclepias*	No	29	0.075	< 0.0001*	< 0.0001*	541.89844	< 0.001*
Subgenus *Podostemma*	No	33	< 0.0001*	< 0.0001*	< 0.0001*	166.16784	0.001*
Subgenus *Asclepiodella*	No	17	0.0052	0.0047	0.0081	82.06040	0.086
Subgenus *Acerates*	No	11	0.028	0.028	0.064	59.97608	0.026
Subgenus *Solanoa*	No	18	0.0020*	0.0020*	0.0041	115.44212	0.014*
Subgenus *Polyotus*	Yes	2	0.62	0.62	1	3.32282	0.97
Subgenus *Asclepiodora*	No	33	< 0.0001*	< 0.0001*	< 0.0001*	164.88379	< 0.001*
Subgenus *Podostigma*	No	12	0.023	0.023	0.052	67.59726	0.19
Series Incarnatae	No	25	0.0001*	< 0.0001*	< 0.0001*	116.23749	0.026*
Series Incarnatae emended	Yes	8	0.046	0.046	0.077	40.54238	0.53
Series Tuberosae	No	2	0.67	0.67	0.83	18.16049	0.86
Series Exaltatae	No	27	0.0002*	0.0002*	0.0001*	142.98725	0.002*
Series Grandiflorae	No	0	1	1	1	0	1
Series Syriacae	No	27	0.0016*	0.0014*	0.0018*	167.59146	0.001*
Series Purpurascentes	No	18	0.0055	0.0056	0.0041	94.11660	0.046*
Series Macrotides	No	36	< 0.0001*	< 0.0001*	< 0.0001*	195.01246	< 0.001*
Series Roseae	No	65	< 0.0001*	< 0.0001*	< 0.0001*	390.72401	< 0.001*
Tests of Geographic Clades							
American species	Yes	2	0.73	0.75	0.69	8.26045	0.96
North American species	No	21	0.0092	0.0048	0.0017*	94.40097	0.074
African species	No	1	0.83	0.83	1	3.36795	1
South American species	Yes	4	0.45	0.31	0.33	28.82195	0.67

In the most densely sampled prior study of *Asclepias* s. l. (55 African and five American species and sequences of the plasid *trnL* intron/*trnL-F* spacer and nuclear ITS), Goyder et al. (2007) found reciprocally monophyletic, sister clades of American and African species. Although the monophyly of *Asclepias* s. s. is well supported in the present study, there is weak evidence that African species may be paraphyletic to American *Asclepias*. With current sampling, it appears that the majority of African species belong to a single clade sister to American *Asclepias*, with only a single African species, *Trachycalymma pseudofimbriatum* falling outside the main African clade (Fig. 2). An unresolved position of *T. pseudofimbriatum* with respect to American and African clades was also found in analyses including dense sampling of African *Asclepias* s. l. (D. Chuba et al. unpubl. data). This species was described recently, but does not stand out as anomalous in *Trachycalymma* (Goyder 2001a). The other sampled species of the genus, *T. buchwaldii* (Schltr. & K. Schum. ex K. Schum.) Goyder is placed in the main clade of African species and was transferred only recently to *Trachycalymma* (Goyder 2001a) from *Asclepias*. Although sampled by Goyder et al. (2007) it does not appear in the published phylogeny (their Fig. 1), but is found in the cpDNA-only tree deposited in TreeBASE (S1650, Tr2576), where it is found in a large, unresolved polytomy at the base of a clade containing all sampled African *Asclepias* s. l. Notably, all species of *Trachycalymma*, including the two sampled here, were transferred to *Asclepias* recently by Goyder (2009). Further sampling of *Trachycalymma* and additional loci are warranted to explore the apparent paraphyly of African *Asclepias* s. l.

Species Paraphyly?—There was strong support for paraphyly of two species for which multiple accessions were included, *A. californica* and *A. tuberosa*. Sequences of *A. californica* subsp. *greenei* were nearly identical to those of *A. vestita* subsp. *vestita*, rendering *A. californica* polyphyletic and *A. vestita* paraphyletic (Fig. 2A). This result is surprising given the morphological homogeneity within these species and several easily characterized floral differences used by Woodson (1954b) to place these species in different subgenera. Possible explanations include introgression of the plastid genome of *A. v.* subsp. *vestita* into *A. c.* subsp. *greenei* and incomplete lineage sorting. Because the sequences of *A. c.* subsp. *greenei* and *A. v.* subsp. *vestita* are nearly identical, introgression is the most plausible hypothesis for non-monophyly, and is supported by patterns of variation in *A. vestita* and *A. californica* ITS sequences (M. Fishbein et al. unpubl. data). Another instance of species paraphyly involves *A. tuberosa* and *A. obovata* (Fig. 2B). Two accessions of *A. tuberosa* subsp. *interior* form a clade with *A. obovata*, whereas the accession of *A. tuberosa* subsp. *rolfsii* is placed in an unresolved position within a larger clade including *A. syriaca*, *A. michauxii* Decne., and *A. rubra* L. Again, the morphological homogeneity of *A. tuberosa* makes paraphyly of the species quite surprising. Of the taxa involved, *A. syriaca* is distributed to the north or west of the range of the remaining species. However, *A. obovata* overlaps in distribution with both sampled subspecies of *A. tuberosa* on the Gulf coastal plain of the U. S. A., where *A. michauxii* and *A. rubra* are also distributed. Like the case of *A. californica* and *A. vestita*, both introgression and incomplete lineage sorting are plausible hypotheses for species non-monophyly. Sequences of multiple nuclear loci analyzed in the context of coalescent theory may be needed to discover the true species phylogeny in these and other portions of the *Asclepias* tree (Maddison and Knowles 2006; Carstens and Knowles 2007; Liu et al. 2008).

Evaluation of Woodson's (1954b) Infrageneric Classification—Although the backbone of the phylogenetic tree of American *Asclepias* s. s. is not well resolved, there are a number

of well supported clades that provide ample evidence that the infrageneric classification into subgenera and series proposed by Woodson (1954b) does not reflect phylogeny. Of the 16 taxa containing more than one species, only one, subgenus *Polyotus*, was present in the MPTs and ML tree. More than one half of the taxa were significantly rejected by parsimony- and likelihood-based tests under conservative sequential Bonferroni correction (Table 5). Many of the remainder would be significantly rejected under conventional *p* values. Thus, substantive revision of the classification of *Asclepias* is warranted.

Subgenus *Asclepias* contains 71 of 107 (66%) of the North American species treated by Woodson (1954b). The monophyly of this subgenus is significantly rejected by two of three parsimony-based tests and the SH test (Table 5). Members of this subgenus are placed in every one of the major clades of American species (clades A-P in Fig. 2), except the wholly South American clade (G). Non-monophyly of subg. *Asclepias* is the result of numerous instances in which members of this taxon are placed in the same well-supported clade with members of other subgenera (e.g. with members of subg. *Podostemma* in clades I, M and N; subg. *Asclepiodora* in clades J and K; subg. *Acerates* in clade N; subg. *Anatherix* and subg. *Podostigma* in clade O; and subg. *Asclepiodella*, subg. *Asclepiodora* and subg. *Podostigma* in clade P). Three other subgenera (subg. *Podostemma*, subg. *Solanoa*, and subg. *Asclepiodora*) were also found to be significantly non-monophyletic (Table 5).

Species of subg. *Podostemma* are placed in clades I, M, and N (Fig. 2). Most are placed in a single clade (M), along with *A. arenaria* of subg. *Ascelpias* and *A. prostrata* W. H. Blackw. *Asclepias prostrata* is morphologically similar to the species of subg. *Podostemma* with which it is placed, and likely would have been included there by Woodson. However, *A. arenaria* does not possess the spatulate corona lobes used by Woodson (1954b) to diagnose subg. *Podostemma*. Nonetheless, *A. arenaria* grows in arid, sandy habitats similar to those of others in clade M, suggesting that floral divergence masked the true affinities of this species. Of the two species of subg. *Podostemma* placed in other clades, *A. subulata* was strongly supported as belonging to clade (I), which contains species from subg. *Asclepias* that share with *A. subulata* robust, essentially leafless habits and a distribution endemic to the Sonoran Desert. Placement of the remaining member of subg. *Podostemma*, *A. auriculata* Kunth, is well supported in one of the two highland Mexican clades (N), concordant with geographic and ecological similarities.

Species of subg. *Asclepiodora* are placed in clades J, K, and P (Fig. 2). Clade J is a morphologically homogeneous group of glaucous-leaved species distributed from Arizona and Texas to Central America treated by Woodson in subg. *Asclepidora* and subg. *Asclepias* ser. Grandiflorae. Although the species are virtually indistinguishable in the absence of flowers, Woodson placed them in different subgenera based on the position of erect (subg. *Asclepias*) versus deflexed (subg. *Asclepiodora*) corona lobes. Clade K is morphologically diverse and contains elements of subg. *Asclepiodora* and ser. Macrotides and ser. Roseae of subg. *Asclepias*.

Subgenus *Solanoa* consists of only three species. *Asclepias californica* is well supported as paraphyletic to *A. vestita* (subg. *Asclepias* ser. Roseae), a species with which it is sympatric in California, whereas *A. cryptoceras* is placed in a subclade with species of ser. Exaltatae and ser. Syriacae of subg. *Asclepias*; these species share arid habitats and glaucous leaves, but are distributed disjunctly. The remaining species of subg. *Solanoa*, *A. solanoana* Woodson, has an unresolved placement in clade D.

None of the other subgenera were significantly rejected as monophyletic. Subg. *Polyotus* was found to be weakly monophyletic and subg. *Anatherix* is monospecific. The remaining subgenera (subg. *Acerates*, subg. *Asclepiodella*, and subg. *Podostigma*) were found to be non-monophyletic, but not significantly so (Fig. 2; Table 5).

Of the eight series of subg. *Asclepias*, the monophyly of five is statistically rejected. The largest of these, series Incarnatae, merits special attention. All but one species (*A. leptopus*) of series Incarnatae is placed in a single clade (F). This clade also includes all sampled South American species of *Asclepias*, which were not treated in Woodson's (1954b) classification. The South American species include some (e.g. *A. mellodora* A. St.-Hil.) that are similar to members of ser. Incarnatae (e.g. *A. curassavica*), but also others (e.g. *A. barjoniifolia* E. Fourn.) with no clear counterpart in that series. Nonetheless, the South American species form a well supported clade (G) within a paraphyletic group of ser. Incarnatae, suggesting the morphological divergence in these species may be related to ecological release following colonization of temperate South America. Although the monophyly of an emended Incarnatae, excluding *A. leptopus* and including the South American species, is strongly supported (BS 100, DI 8, PP 1), non-monophyly of this clade cannot be rejected statistically (Table 5).

The monophyly of ser. Exaltatae, ser. Syriacae, ser. Macrotides, and ser. Roseae were statistically rejected. These taxa each contain species placed in the highland Mexican clade (E), and the northern temperate American clade (D). Within clade D, each of these series includes species placed in the eastern U. S. A. clade (L) along with members of ser. Tuberosae. Series Macrotides and ser. Roseae include species in a second northern temperate clade (K) along with species of subg. *Asclepiodora*. Series Roseae includes a species (*A. arenaria*) that is strongly supported as belonging to a clade (M) containing mostly species of subg. *Podostemma*. Series Roseae also includes most of the species that comprise the leafless, Sonoran Desert clade (I). Although not statistically significant, the monophyly of ser. Purpurascentes costs 18 extra steps under parsimony and is over 94 likelihood units less likely. This taxon, too, has species placed in the eastern U. S. A. clade (L), elsewhere in the north temperate clade (D), and in the highland Mexican clade (E). The small series Tuberosae and Grandiflorae each consist of species that are closely related and may eventually prove to be monophyletic, but the currently available data are not decisive. Overall, it is difficult to generalize as to why Woodson's (1954b) classification corresponds so poorly to phylogenetic relationships. One recurring theme, however, is that he seems to have overemphasized floral morphology, particularly corona structure, to the complete exclusion of vegetative morphology.

Towards a Revised Infrageneric Classification of Asclepias— Although the monophyly of the majority of Woodson's infrageneric taxa are rejected by statistical hypothesis tests, and most of the rest are quite likely not monophyletic, some of these taxa contain monophyletic cores that could be revived through taxonomic revision. Such revision is not formally proposed here, due to the lack of phylogenetic resolution among major clades and sequence data that is limited to the plastid genome. Full resolution of the plastid phylogeny appears promising using complete genome sequences

(S. Straub, Oregon State University et al. unpubl. data). Here, we highlight strongly supported clades that are likely to be part of the future taxonomy of *Asclepias*.

Major clades diverging near the root of *Asclepias* s. s. include the temperate North American clade (D in Fig. 2), the highland Mexican clade (E), a clade (F) consisting largely of members of subg. *Asclepias* ser. Incarnatae (see Fig. 1D), and the Sonoran Desert clade (I; see Fig. 1E). Within clade D, well-supported clades that may merit taxonomic recognition include clades J, K, L, M, and O. Clade J is morphologically homogeneous (tall with broad, glaucous leaves) and contains species found primarily in montane Mexico (see Fig. 1F). Clade K is morphologically disparate with several species distributed on the Colorado Plateau of the southwestern U. S. A. and adjacent areas (see Fig. 1G). Clade L is also morphologically diverse and the species, except *A. hallii* A. Gray of the Rocky Mountains, are distributed throughout the eastern U. S. A. and Canada (see Fig. 1H). Clade M is fairly homogeneous morphologically (sessile inflorescences, spatulate corona lobes) and contains all species of subg. *Podostemma*, except *A. subulata*, plus *A. arenaria* (see Fig. 1I). This is one of the few examples of a Woodsonian taxon (Woodson 1954b) that can be retained with slight modification in a classification that reflects phylogeny. The small clade O is morphologically disparate, including the sole member of subg. *Anatherix*, *A. connivens*. However, the three species are narrowly distributed in pine flatwoods in northern Florida and the Atlantic coastal plain (see Fig. 1K). Within clade E, there are two well-supported subclades that may merit taxonomic recognition. Clade N was well supported by both BS and PP and consists of Mexican highland species that are mostly broad-leaved (see Fig. 1J) and were classified in various series of subg. *Asclepias* by Woodson (1954b). Clade P was only well supported by PP and consists of both broad-leaved and narrow-leaved Mexican highland species spanning four of Woodson's subgenera (see Fig. 1L).

Implications of the Phylogeny of Asclepias for Comparative Analyses—Broad interest in the evolutionary ecology of milkweeds, especially involving interspecific interactions with arthropods, has stimulated the desire to use a phylogenetic framework to conduct rigorous analyses of evolutionary hypotheses. In the absence of an explicit phylogeny, Farrell and Mitter (1998) used an interpretation of Woodson's (1954b) infrageneric classification to test a hypothesis of cospeciation between *Asclepias* and a specialist coleopteran herbivore, *Tetraopes*. The rampant non-monophyly of Woodson's taxa provides the impetus for a fresh look at this question; however, the poor resolution among major clades found in the present study suggests that such a reanalysis may need to wait for the completion of additional phylogenetic work. In a recent series of papers, Agrawal, Fishbein, and colleagues (Agrawal and Fishbein 2006, 2008; Agrawal et al. 2008; Agrawal et al. 2009a, b) used phylogenetically explicit analyses incorporating subsets of the data presented here to study correlations and trends in the evolution of defense-related traits, such as the quantity of latex, the quantity of cardenolides, the quantity and diversity of phenolics, and the density of leaf trichomes. These studies found that results were rarely sensitive to phylogenetic uncertainty engendered by the lack of resolution among major clades, although some evolutionary correlations did depend on particular topologies (Agrawal and Fishbein 2006). We anticipate that the emerging picture of the phylogenetic history of *Asclepias* coming into focus in this contribution will spur comparative ecological and evolutionary studies of these fascinating plants.

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Appendix 1. Provenance and voucher data for accessions of *Asclepias* and outgroups used as sources for DNA sequences. GenBank accession numbers are provided for each sequence. Data presented in the following format: taxon, infrageneric taxon (for North American species only) following Woodson (1954b): provenance, voucher (acronym of herbarium deposition; Holmgren et al. 1990), *rpl16* intron accession number, *trnC–rpoB* spacer accession number, *trnS–G* spacer/*trnG* intron accession number. Woodson's (1954b) infrageneric taxa indicated as follows: 1a: subg. *Asclepias*, ser. Incarnatae; 1b: subg. *Asclepias*, ser. Tuberosae; 1c: subg. *Asclepias*, ser. Exaltatae; 1d: subg. *Asclepias*, ser. Grandiflorae; 1e: subg. *Asclepias*, ser. Syriacae; 1f: subg. *Asclepias*, ser. Purpurascentes; 1g: subg. *Asclepias*, ser. Macrotides; 1h: subg. *Asclepias*, ser. Roseae; 2: subg. *Podostemma*; 3: subg. *Anatherix*; 4: subg. *Asclepiodella*; 5: subg. *Acerates*; 6: subg. *Solanoa*; 7: subg. *Polyotus*; 8: subg. *Asclepiodora*; 9: subg. *Podostigma*; NA: species not recognized by Woodson (1954b) or described after 1954.

***North American Asclepias*—*Asclepias albicans* S. Watson, 1h:** U. S. A., California, *Lynch 10715* (LSUS), GQ281153, GQ304302, GQ304419. ***Asclepias albicans* x *subulata*, NA:** Mexico, Baja California Sur, *Fishbein 3142* (WS), GQ281151, GQ304300, GQ304417. ***Asclepias amplexicaulis* Sm., 1c:** U. S. A., Wisconsin, *Lynch 12652* (LSUS), GQ281212, GQ304361, GQ304477. ***Asclepias angustifolia* Schweigg., 1a:** Mexico, Sonora, *Fishbein 3678* (WS), EU675531, EU675569, EU675607. ***Asclepias arenaria* Torr., 1h:** U. S. A., New Mexico, *Lynch 11495* (LSUS), GQ281158, GQ304307, GQ304424. ***Asclepias asperula* (Decne.) Woodson ssp. *asperula*, 8:** U. S. A., Texas, *Lynch 12014* (LSUS), EU675540, EU675578, EU675616. ***Asclepias asperula* (Decne.) Woodson ssp. *capricornu* (Woodson) Woodson, 8:** U. S. A., Texas, *Lynch 13314* (LSUS), GQ281230, GQ304379, GQ304494. ***Asclepias atroviolacea* Woodson, 9:** Mexico, Sonora, *Fishbein 3612* (ARIZ), GQ281169, GQ304318, GQ304435. ***Asclepias auriculata* Kunth, 2:** Mexico, Nayarit, *Steinmann 1077* (ARIZ), GQ281193, GQ304342, GQ304459. ***Asclepias brachystephana* Engelm. ex Torr., 4:** U. S. A., Texas, *Lynch 10642* (LSUS), GQ281191, GQ304340, GQ304457. ***Asclepias californica* Greene ssp. *californica*, 6:** U. S. A., California, *Lynch 10779* (LSUS), EU675514, EU675552, EU675590. ***Asclepias californica* Greene ssp. *greenei* Woodson, 6:** U. S. A., California, *Lynch 10820* (LSUS), GQ281164, GQ304313, GQ304430. ***Asclepias cinerea* Walter, 4**: U. S. A., Florida, *Fishbein 4793* (OKLA), GQ281240, GQ304389, GQ304504. ***Asclepias circinalis* (Decne.) Woodson, 9:** Mexico, Michoacán, *Fishbein 5179* (OKLA), GQ281197, GQ304346, GQ304462. ***Asclepias connivens* Baldwin ex Elliott, 3:** U. S. A., Florida, *Lynch 12336* (LSUS), GQ281225, GQ304374, GQ304489. ***Asclepias cordifolia* (Benth.) Jeps., 4:** U. S. A., California, *Lynch 10942* (LSUS), EU675518, EU675556, EU675594. ***Asclepias coulteri* A. Gray, 1c:** Mexico, Querétaro, *Fishbein 5172* (OKLA), GQ281201, GQ304350, GQ304466. ***Asclepias cryptoceras* S. Watson ssp. *cryptoceras*, 6:** U. S. A., Colorado, *Weber et al. 3133* (WS), EU675516, EU675554, EU675592. ***Asclepias cryptoceras* S. Watson ssp. *davisii* (Woodson) Woodson, 6:** U. S. A., California, *Lynch 10995* (LSUS), GQ281167, GQ304316, GQ304433. ***Asclepias curassavica* L., 1a:** U. S. A., Florida, *Lynch 12542* (LSUS), EU675522, EU675560, EU675598. ***Asclepias curtissii* A. Gray, 1f:** U. S. A., Florida, *Lynch 12461*, (LSUS), GQ281217, GQ304366, GQ304482. ***Asclepias cutleri* Woodson, 4:** U. S. A., Utah, *Lynch 12112* (LSUS), GQ281149, GQ304298, GQ304415. ***Asclepias elata* Benth., 8:** U. S. A., Arizona, *Lynch 11410* (LSUS), GQ281235,

GQ304384, GQ304499. ***Asclepias elegantula*** **Fishbein, NA:** Mexico, Querétaro, *Fishbein 5153* (ARIZ), GQ281202, GQ304351, GQ304467. ***Asclepias emoryi*** **(Greene) Vail ex Small, 2:** U. S. A., Texas, *Carr 12032* (TEX), GQ281155, GQ304304, GQ304421. ***Asclepias engelmanniana*** **Woodson, 7:** U. S. A., Kansas, *Lynch 11224* (LSUS), EU675511, EU675549, EU675587. ***Asclepias eriocarpa*** **Torr., 1h:** U. S. A., California, *Lynch 10923* (LSUS), EU675542, EU675580, EU675618. ***Asclepias erosa*** **Torr., 1h:** U. S. A., California, *Hufford 3593* (OKLA), EU675515, EU675553, EU675591. ***Asclepias exaltata*** **L., 1c:** U. S. A., Ohio, *Lynch 12823* (LSUS), EU675536, EU675574, EU675612. ***Asclepias fascicularis*** **Decne., 1a:** U. S. A., California, *Fishbein 4401* (WS), EU675523, EU675561, EU675599. ***Asclepias feayi*** **Chapm. ex A. Gray, 4:** U. S. A., Florida, *Fishbein 5586* (MISSA), GQ281239, GQ304388, GQ304503. ***Asclepias fournieri*** **Woodson, 8:** Mexico, Sonora, *Fishbein 3660* (ARIZ), GQ281190, GQ304339, GQ304456. ***Asclepias gentryi*** **Standl., 1a:** Mexico, Sonora, *Fishbein 3622* (MEXU), GQ281210, GQ304359, GQ304475. ***Asclepias glaucescens*** **Kunth, 1d:** Mexico, Oaxaca, *Lynch 14142* (LSUS), GQ281234, GQ304383, GQ304498. ***Asclepias*** **aff. *glaucescens*, NA:** Mexico, Sonora, *Fishbein 3671* (ARIZ), EU675543, EU675581, EU675619. ***Asclepias hallii*** **A. Gray, 1f:** U. S. A., Colorado, *Lynch 11304* (LSUS), EU675534, EU675572, EU675610. ***Asclepias hirtella*** **(Pennell) Woodson, 5:** U. S. A., Wisconsin, *Lynch 12700* (LSUS), EU675512, EU675550, EU675588. ***Asclepias humistrata*** **Walter, 1e:** U. S. A., Florida, *Lynch 12311* (LSUS), EU675517, EU675555, EU675593, ***Asclepias hypoleuca*** **(A. Gray) Woodson, 1e:** U. S. A., Arizona, *Lynch 11374* (LSUS), GQ281171, GQ304320, GQ304437. ***Asclepias incarnata*** **L. ssp. *incarnata*, 1a:** U. S. A., Wisconsin, *Lynch 12567* (LSUS), EU675529, EU675567, EU675605. ***Asclepias incarnata*** **L. ssp. *pulchra*** **(Ehrh. ex Willd.) Woodson, 1a:** U. S. A., Connecticut, *Bissett 2601* (ARIZ), EU675530, EU675568, EU675606. ***Asclepias involucrata*** **Englem. ex Torr., 1g:** U. S. A., Arizona, *Lynch 12050* (LSUS), GQ281228, GQ304377, GQ304492. ***Asclepias jaliscana*** **B. L. Rob., 1c:** Mexico, Sonora, *Fishbein 3657* (WS), GQ281203, GQ304352, GQ304468. ***Asclepias jorgeana*** **Fishbein & S. P. Lynch. NA:** Mexico, Durango, *Lynch 14175* (LSUS), GQ281194, GQ304343, GQ304460. ***Asclepias labriformis*** **M. E. Jones, 1h:** U. S. A., Utah, *Lynch 11344* (LSUS), GQ281229, GQ304378, GQ304493. ***Asclepias lanceolata*** **Walter, 1b:** U. S. A., Florida, *Fishbein 2415* (ARIZ), GQ281213, GQ304362, GQ304478. ***Asclepias latifolia*** **(Torr.) Raf., 1h:** U. S. A., New Mexico, *Lynch 11014* (LSUS), EU675532, EU675570, EU675608. ***Asclepias lemmonii*** **A. Gray, 1g:** U. S. A., Arizona, *Lynch 11453* (LSUS) GQ281177, GQ304326, GQ304443. ***Asclepias leptopus*** **I. M. Johnst., 1a:** Mexico, Sonora, *Fishbein 3589* (WS), GQ281150, GQ304299, GQ304416. ***Asclepias linaria*** **Cav., 1e:** U. S. A., Arizona, *Lynch 11386* (LSUS), GQ281168, GQ304317, GQ304434. ***Asclepias linearis*** **Scheele, 1a:** U. S. A., Texas, *Fryxell 2949* (TEX), GQ281208, GQ304357, GQ304473. ***Asclepias longifolia*** **Michx., 5:** U. S. A., Florida, *Lynch 12447* (LSUS), GQ281161, GQ304310, GQ304427. ***Asclepias lynchiana*** **Fishbein, 1d:** Mexico, Guerrero, *Venable & Becerra s. n.* (ARIZ), GQ281237, GQ304386, GQ304501. ***Asclepias macrosperma*** **Eastw., 1g:** U. S. A., Arizona, *Gierisch 4191* (ARIZ), GQ281227, GQ304376, GQ304491. ***Asclepias macrotis*** **Torr., 1g:** U. S. A., New Mexico, *Lynch 11260* (LSUS), GQ281200, GQ304349, GQ304465. ***Asclepias macroura*** **A. Gray, NA:** Mexico, Durango, *Lynch 14166* (LSUS), GQ281238, GQ304387, GQ304502. ***Asclepias masonii*** **Woodson, 1h:** Mexico, Baja California Sur, *Fishbein 3101* (WS), GQ281152, GQ304301, GQ304418. ***Asclepias mcvaughii*** **Woodson, 9:** Mexico, Jalisco *McVaugh 22013* (ARIZ), GQ281170, GQ304319, GQ304436. ***Asclepias meadii*** **Torr. ex A. Gray, 1e:** U. S. A., Kansas, *Freeman 9106* (KANU), GQ281218, GQ304367, GQ304483. ***Asclepias melantha*** **Decne., 1g:** Mexico, Oaxaca, *Fishbein 5837* (OKLA), GQ281172, GQ304321, GQ304438. ***Asclepias mexicana*** **Cav., 1a:** Mexico, Nuevo Leon, *Fishbein 3009* (ARIZ), GQ281209, GQ304358, GQ304474. ***Asclepias michauxii*** **Decne., 1g:** U. S. A., Florida, *Lynch 12316* (LSUS), GQ281223, GQ304372, GQ304487. ***Asclepias mirifica*** **Woodson, 8:** Mexico, Sonora, *Fishbein 3627* (ARIZ), GQ281236, GQ304385, GQ304500. ***Asclepias nivea*** **L., 1a:** Dominican Republic, Barahona, *Moody 46* (WS), EU675521, EU675559, EU675597. ***Asclepias notha*** **W. D. Stevens, NA:** Mexico, Tlaxcala, *Lynch 14113* (LSUS), GQ281180, GQ304329, GQ304446. ***Asclepias*** **sp. nov. cf. *notha*, NA:** Mexico, Oaxaca, *Fishbein 5816* (OKLA), GQ281181, GQ304330, GQ304447. ***Asclepias nummularia*** **Torr., 1h:** U. S. A., Arizona, *Lynch 11439* (LSUS), GQ281176, GQ304325, GQ304442. ***Asclepias nyctaginifolia*** **A. Gray, 2:** U. S. A., Arizona, *Fishbein 2445* (ARIZ), EU675510, EU675548, EU675586. ***Asclepias obovata*** **Elliott, 1h:** U. S. A., Florida, *Lynch 12222* (LSUS), GQ281222, GQ304371, GQ304486. ***Asclepias oenotheroides*** **Schltdl. & Cham., 2:** U. S. A., Texas, *Lynch 13339* (LSUS), EU675509, EU675547, EU675585. ***Asclepias otarioides*** **E. Fourn., 1f:** Mexico, Sinaloa, *Bellsey 97-5* (ARIZ), GQ281178, GQ304327, GQ304444. ***Asclepias ovalifolia*** **Decne., 1e:** U. S. A., South Dakota, *Lynch 13546* (LSUS), GQ281215, GQ304364, GQ304480. ***Asclepias ovata*** **M. Martens & Galeotti, 1c:** Mexico, Chihuahua, *Laferrière 1478* (MO), GQ281179, GQ304328, GQ304445. ***Asclepias pedicellata*** **Walter, 9:** U. S. A., Florida, *Fishbein 5217* (OKLA), GQ281242, GQ304391, GQ304506. ***Asclepias pellucida*** **E. Fourn., 1e:** Mexico, Jalisco, *Wetter 2005* (WIS), GQ281192, GQ304341, GQ304458. ***Asclepias perennis*** **Walter, 1a:** U. S. A., Florida, *Lynch 12408* (LSUS), EU675528, EU675566, EU675604. ***Asclepias pratensis*** **Benth., 1e:** Mexico, Guanajuato, *Fishbein 5143* (OKLA), GQ281199, GQ304348, GQ304464. ***Asclepias pringlei*** **(Greenm.) Woodson, 1e:** Mexico, Distrito Federal, *Ventura 2807* (ARIZ), GQ281195, GQ304334,–. ***Asclepias*** **aff. *pringlei*, NA:** Mexico, Michoacán, *Fishbein 5119* (OKLA), GQ281196, GQ304345, GQ304461. ***Asclepias*** **sp. nov. cf. *pringlei*, NA:** Mexico, Querétaro, *Fishbein 5175* (OKLA), GQ281182, GQ304331, GQ304448. ***Asclepias prostrata*** **W. H. Blackw., NA:** U. S. A., Texas, *Fishbein 2432* (ARIZ), GQ281159, GQ304308, GQ304425. ***Asclepias puberula*** **A. Gray, 1g:** Mexico, Jalisco, *Vásquez 7332* (OKLA), GQ281198, GQ304347, GQ304463. ***Asclepias pumila*** **(A. Gray) Vail, 1a:** U. S. A., New Mexico, *Lynch 11236* (LSUS), EU675525, EU675563, EU675601. ***Asclepias purpurascens*** **L., 1f:** U. S. A., Ohio, *Lynch 12847* (LSUS), EU675535, EU675573, EU675611. ***Asclepias quadrifolia*** **Jacq., 1e:** U. S. A., Tennessee, *Fishbein 5330* (MISSA), GQ281219, GQ304368, GQ304484. ***Asclepias quinquedentata*** **A. Gray, 1c:** U. S. A., Arizona, *Fishbein 2850* (ARIZ), GQ281174, GQ304323, GQ304440. ***Asclepias rosea*** **Kunth, 1h:** Mexico, Michoacán, *Steinmann 4304* (OKLA), GQ281173, GQ304322, GQ304439. ***Asclepias rubra*** **L., 1b:** U. S. A., Louisiana, *Fishbein 5199* (OKLA), GQ281243, GQ304392, GQ304507. ***Asclepias ruthiae*** **Maguire, 4:** U. S. A., Arizona, *Lynch 12068* (LSUS), GQ281231, GQ304380, GQ304495. ***Asclepias sanjuanensis*****, K. D. Heil, J. M. Porter & S. L. Welsh, NA:** U. S. A., New Mexico, *Ellison s. n.* (OKLA), GQ281233, GQ304382, GQ304497. ***Asclepias scaposa*** **Vail, 1c:** Mexico, Nuevo Leon, *Fishbein 2951* (ARIZ), GQ281162, GQ304311, GQ304428. ***Asclepias schaffneri*** **A. Gray, NA:** Mexico, Zacatecas, *Fishbein 5846* (OKLA), GQ281175, GQ304324, GQ304441. ***Asclepias scheryi*** **Woodson, NA:** Mexico, Jalisco, *Fishbein 5137* (OKLA), GQ281185, GQ304334, GQ304451; Mexico, Michoacán, *Zamudio 5234* (IEB), GQ281186, GQ304335, GQ304452. ***Asclepias similis*** **Hemsl., 1c:** Mexico, Nuevo Leon, *Fishbein 3000* (ARIZ), GQ281187, GQ304336, GQ304453; Mexico, Querétaro, *Fishbein 5148* (MISSA), GQ281188, GQ304337, GQ304454. ***Asclepias solanoana*** **Woodson, 6:** U. S. A., California, *Lynch 10884* (LSUS), GQ281163, GQ304312, GQ304429. ***Asclepias speciosa*** **Torr., 1f:** U. S. A., California, *Gendron s. n.* (OKLA), GQ281214, GQ304363, GQ304479; U. S. A., New Mexico, *Lynch 11492* (LSUS), EU675533, EU675571, EU675609. ***Asclepias standleyi*** **Woodson, 2:** Mexico, Sonora, *Van Devender 93-214* (ARIZ), GQ281157, GQ304306, GQ304423. ***Asclepias*** **aff. *standleyi*, NA:** Mexico, Sonora, *Reina 98-579* (WS), GQ281156, GQ304305, GQ304422. ***Asclepias stenophylla*** **A. Gray, 7:** U. S. A., Colorado, *Fishbein 5394* (OKLA),–,–, GQ304426; U. S. A., Kansas, *Fishbein 2817* (ARIZ), GQ281160, GQ304309,–. ***Asclepias subaphylla*** **Woodson, 1h:** Mexico, Sonora, *Fishbein 3518* (WS), GQ281154, GQ304303, GQ304420. ***Asclepias subulata*** **Decne., 2:** Mexico, Baja California Sur, *Fishbein 3081* (WS), EU675508, EU675546, EU675584. ***Asclepias subverticillata*** **(A. Gray) Vail, 1a:** Mexico, Nuevo Leon, *Fishbein 2948* (ARIZ), GQ281207, GQ304356, GQ304472; U. S. A., New Mexico, *Lynch 11012* (LSUS), EU675526, EU675564, EU675602. ***Asclepias sullivantii*** **Torr., 1f:** U. S. A., Kansas, *Lynch 11147* (LSUS), EU675539, EU675577, EU675615. ***Asclepias syriaca*** **L., 1e:** U. S. A., Wisconsin, *Lynch 12589* (LSUS), EU675537, EU675575, EU675613. ***Asclepias texana*** **A. Heller, 1a:** U. S. A., Texas, *Fishbein 2404* (ARIZ), EU675527, EU675565, EU675603. ***Asclepias tomentosa*** **Elliott, 1h:** U. S. A., Florida, *Fishbein 2419* (ARIZ),–, GQ304373,–; *Fishbein 5608* (MISSA), GQ281224,–, GQ304488. ***Asclepias tuberosa*** **L. ssp.** *interior* **Woodson, 1b:** U. S. A., Kansas, *Fishbein 2816* (ARIZ), GQ281221, GQ304370,–; U. S. A., Mississippi, *Fishbein 4825* (MISSA), EU675538, EU675576, EU675614. ***Asclepias tuberosa*** **L. ssp. *rolfsii*** **(Britton ex Vail) Woodson, 1b:** U. S. A., Florida, *Lynch 12526* (LSUS), GQ281220, GQ304369, GQ304485. ***Asclepias uncialis*** **Greene, 4:** U. S. A., Arizona, *Porter 7021* (ARIZ), GQ281232, GQ304381, GQ304496. ***Asclepias variegata*** **L., 1f:** U. S. A., Ohio, *Lynch 12787* (LSUS), GQ281216, GQ304365, GQ304481. ***Asclepias verticillata*** **L., 1a:** U. S. A., Kansas, *Lynch 11102* (LSUS), EU675524, EU675562, EU675600. ***Asclepias vestita*** **Hook. & Arn. ssp. *parishii*** **(Jeps.) Woodson, 1h:** U. S. A., California, *Lynch 10735* (LSUS), GQ281165, GQ304314, GQ304431. ***Asclepias vestita*** **Hook. & Arn. ssp. *vestita*, 1h:** U. S. A., California, *Lynch 10854* (LSUS), GQ281166, GQ304315, GQ304432. ***Asclepias vinosa*** **(E. Fourn.) Woodson, 5:** Mexico, Morelos, *Rzedowski 38150* (ARIZ), GQ281183, GQ304332, GQ304449. ***Asclepias viridiflora*** **Raf., 5:** U. S. A., Ohio, *Lynch 12865* (LSUS), EU675513, EU675551, EU675589. ***Asclepias viridis*** **Walter, 8:** U. S. A., Florida, *Lynch 12491* (LSUS), EU675541, EU675579, EU675617. ***Asclepias viridula*** **Chapm., 1e:** U. S. A., Florida, *Fishbein 4806* (MISSA), GQ281241, GQ304390, GQ304505. ***Asclepias virletii*** **E. Fourn., 1c:** Mexico, Nuevo Leon, *Hinton 18172* (TEX), GQ281184, GQ304333, GQ304450. ***Asclepias welshii*** **N. H. Holmgren & P. K. Holmgren, NA:** U. S. A., Utah, *Lynch 11369* (LSUS), GQ281226, GQ304375, GQ304490. ***Asclepias woodsoniana*** **Standl. & Steyerm., 1a:**

Nicaragua, *Neil 242* (MO), GQ281211, GQ304360, GQ304476. ***Asclepias zanthodacryon* (L. B. Sm.) Woodson, 8:** Mexico, Nuevo Leon, *Fishbein 3010* (ARIZ), GQ281189, GQ304338, GQ304455.

*South American Asclepias—**Asclepias barjoniifolia* **E. Fourn.:** Bolivia, Tarija, *Wood 9532* (K), EU675520, EU675558, EU675596. ***Asclepias boliviensis* E. Fourn.:** Bolivia, Santa Cruz, *Wood 11724* (K), EU675519, EU675557, EU675595. ***Asclepias candida* Vell.:** Bolivia, Santiago, *Wood & Goyder 16919* (K), GQ281204, GQ304353, GQ304469. ***Asclepias flava* (Kuntze) Lillo, non N. E. Br.:** Argentina, *Zuloaga & Morrone 7215* (OKLA), GQ281206, GQ304355, GQ304471. ***Asclepias mellodora* A. St.-Hil. var.** *mellodora***:** Argentina, *Zuloaga & Morrone 7069* (OKLA), GQ281205, GQ304354, GQ304470.

African Asclepias s. l.—Asclepias aurea* (Schltr.) Schltr.:** South Africa, Transvaal, *Germishuizen 2107* (K), GQ281142, GQ304291, GQ304408. ***Asclepias densiflora* N. E. Br.:** South Africa, *Balkwill 10844* (J), GQ281148, GQ304297, GQ304414. ***Asclepias gibba* Schltr.:** Lesotho, Makhalaneng, *Hargreaves 3483* (K), GQ281143, GQ304292, GQ304409. ***Asclepias macropus* (Schltr.) Schltr.:** South Africa, Cape, *Bester 3582* (K), GQ281144, GQ304293, GQ304410. ***Asclepias multicaulis* (E. Mey.) Schltr., non Vellozo:** South Africa, Transkei, *Hilliard & Burt 18708* (K), GQ281147, GQ304296, GQ304413. ***Asclepias praemorsa* Schltr.:** South Africa, Transkei, *Nicholas & Smook 2388* (K), GQ281145, GQ304294, GQ304411. ***Asclepias randii* S. Moore:** Tanzania, Mbeya, *Goyder 3854* (K), GQ281146, GQ304295, GQ304412. ***Glossostelma carsonii* (N. E. Br.) Bullock:** Malawi, Northern Region, *Phillips 4166B* (MO), GQ281132, GQ304280, GQ304397. ***Gomphocarpus cancellatus* (Burm. f.) Bruyns:** South Africa, *Drewe 534* (K), EU675507, EU675545, EU675583. ***Gomphocarpus fruticosus* (L.) W. T. Aiton ssp.** *fruticosus:** France, Corsica, *Lambinon 95/493* (ARIZ), EU675506, EU675544, EU675582. ***Gomphocarpus fruticosus* (L.) W. T. Aiton ssp.** *rostratus* **(N. E. Br.) Goyder & Nicholas:** Botswana, *Long & Rae 491* (K), GQ281136, GQ304284, GQ304401. ***Margaretta rosea* Oliv. spp.** *corallina* **Goyder:** Tanzania, Ufipa Dist., *Goyder et al. 3791* (K, type),–, GQ304290, GQ304407. ***Pachycarpus lineolatus* (Decne.) Bullock:** Tanzania, Bukoba Dist., *Gereau 6290* (MO), GQ281133, GQ304281, GQ304398. ***Schizoglossum alpestre* K. Schum.:** Tanzania, Iringa Dist., *Goyder et al. 3892* (K), GQ281141, GQ304289, GQ304406. ***Stathmostelma gigantiflorum* K. Schum.:** Kenya, Machakos Dist., *Harvey & Vollesen 64* (K), GQ281140, GQ304288, GQ 304405. ***Stenostelma corniculatum* (E. Mey.) Bullock:** South Africa, *Balkwill 10908* (J), GQ281137, GQ304285, GQ304402. ***Trachycalymma buchwaldii* (Schltr. & K. Schum. ex K. Schum.) Goyder:** Tanzania, Iringra Dist., *Goyder 3924* (K), GQ281139, GQ304287, GQ304404. ***Trachycalymma pseudofimbriatum* Goyder:** Ethiopia, Sidamo Prov., *Haugen 1503* (K, type), GQ281138, GQ304286, GQ304403. ***Xysmalobium fraternum* N. E. Br.:** Tanzania, Sumbawanga Dist., *Bidgood et al. 2390* (K), GQ281134, GQ304282, GQ304399. ***Xysmalobium undulatum* (L.) W. T. Aiton:** South Africa, *Balkwill 10846* (J), GQ281135, GQ304283, GQ304400.

***Outgroup—Calotropis procera* (Aiton) W. T. Aiton:** U. S. A., Arizona, *Fishbein 5427* (OKLA), GQ281131, GQ304279, GQ304396. ***Cynanchum ligulatum* (Benth.) Woodson:** Mexico, Sonora, *Fishbein 3581* (WS), GQ281128, GQ304276,–; *Fishbein 3695* (WS),–,–, GQ304393. ***Kanahia laniflora* (Forssk.) R. Br.:** Tanzania, Iringa Dist., *Goyder et al. 3931* (K), GQ281130, GQ304278, GQ304395. ***Pergularia daemia* (Forssk.) Chiov.:** Tanzania, *Fishbein 5445* (OKLA), GQ281129, GQ304277, GQ304394.