Available online at www.sciencedirect.com
ScienceDirect
Molecular Phylogenetics and Evolution xxx (2007) xxx–xxx
MOLECULAR PHYLOGENETICS AND EVOLUTION
www.elsevier.com/locate/ympev

Phylogeny of North American fireflies (Coleoptera: Lampyridae): Implications for the evolution of light signals

Kathrin F. Stanger-Hall [a,*], James E. Lloyd [b], David M. Hillis [a]

[a] *Section of Integrative Biology and Center for Computational Biology and Bioinformatics, School of Biological Sciences, University of Texas at Austin, 1 University Station C0930, Austin, TX 78712-0253, USA*
[b] *Department of Entomology and Nematology, University of Florida, Gainesville, FL 32601-0143, USA*

Received 3 November 2006; revised 24 April 2007; accepted 9 May 2007

Abstract

Representatives of the beetle family Lampyridae (''fireflies'', ''lightningbugs'') are well known for their use of light signals for species recognition during mate search. However, not all species in this family use light for mate attraction, but use chemical signals instead. The lampyrids have a worldwide distribution with more than 2000 described species, but very little is known about their phylogenetic relationships. Within North America, some lampyrids use pheromones as the major mating signal whereas others use visual signals such as extended glows or short light flashes. Here, we use a phylogenetic approach to illuminate the relationships of North American lampyrids and the evolution of their mating signals. Specifically, to establish the first phylogeny of all North American lampyrid genera, we sequenced nuclear (18S) and mitochondrial (16S and COI) genes to investigate the phylogenetic relationships of 26 species from 16 North American (NA) genera and one species from the genus *Pterotus* that was removed recently from the Lampyridae. To test the monophyly of the NA firefly fauna we sequenced the same genes from three European lampyrids and three Asian lampyrids, and included all available Genbank data (27 additional Asian lampyrids and a former lampyrid from Asia, *Rhagophthalmus*). Our results show that the North American lampyrids are not monophyletic. Different subgroups are closely related to species from Europe, Asia and tropical America, respectively. The present classification of fireflies into subfamilies and tribes is not, for the most part, supported by our phylogenetic analysis. Two former lampyrid genera, *Pterotus* and *Rhagophthalmus*, which have recently been removed from this family, are in fact nested within the Lampyridae. Further, we found that the use of light as a sexual signal may have originated one or four times among lampyrids, followed by nine or four losses, respectively. Short flashes originated at least twice and possibly three times independently among our study taxa. The use of short flashes as a mating signal was replaced at least once by the use of long glows, and light signals as mating signals were lost at least three times in our study group and replaced by pheromones as the main signal mode.

Keywords: Lampyrids; Phylogeny; Signal mode; Pheromones; Glows; Flashes; Bayesian analysis; *Aspisoma*; *Bicellonycha*; *Brachylampis*; *Ellychnia*; *Lamprohiza*; *Lampyris*; *Lucidota*; *Lychnuris*; *Micronaspis*; *Microphotus*; *Paraphausis*; *Phausis*; *Phosphaenus*; *Photinus*; *Photuris*; *Pleotomodes*; *Pleotomus*; *Pollaclassis*; *Pterotus*; *Pyractomena*; *Pyropyga*; Cantharidae; Lampyridae; Lycidae; Rhagophthalmidae; 18S; 16S; COI

1. Introduction

In 1966, 1891 lampyrid species in seven subfamilies and 92 genera were listed by McDermott (1966) in his revision of E. Olivier's Lampyridae Catalog of 1910. Today, the number of described lampyrid species exceeds 2000 in more than 100 genera, and perhaps four times this number remain to be described (see Viviani, 2001; Lloyd, 2002). The approximately 120 species (Lloyd, 1997) of described North American (NA) lampyrids seem to be descendents of several invasion events (McDermott, 1964), and are presently classified into four or five subfamilies: Lampyrinae, Photurinae, Ototretinae, and Cyphonocerinae, with the status of Pterotinae (genus *Pterotus*; McDermott, 1964; Crowson, 1972; Lawrence and Newton, 1995)

* Corresponding author. Present address: Department of Plant Biology, University of Georgia at Athens, Athens, GA 30602, USA. Fax: +1 706 542 1695.
E-mail address: ksh@uga.edu (K.F. Stanger-Hall).

1055-7903/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.ympev.2007.05.013

recently being questioned, and *Pterotus* placed in Elateroidea *incertae sedis* (Branham and Wenzel, 2001). Within these subfamilies, 16 genera (including *Pterotus*) are distinguished (McDermott, 1964). Two additional genera, *Tenaspis* and *Aspisoma*, are occasionally found, possibly as accidental migrants from Mexico and Central America (Lloyd, 2002; Fig. 1).

1.1. Classification and phylogenetic relationships of lampyrids

The classification of lampyrids (''fireflies'', ''lightning-bug'') has been pragmatic. It is based on morphological characteristics that were deemed appropriate for a logical organization of the complex diversity observed in this still relatively poorly known group (see McDermott, 1964). Over its history, modifications in classification were due either to the addition of new taxa or to putting emphasis on different morphological characteristics, some of which may have been more reliable for classification than others (McDermott, 1964). Frank McDermott, the preeminent scholar of this group, emphasized that lampyrid classification ''should not be construed as indicating phylogenetic relationships'' (McDermott, 1964, p. 6). Likewise, Crowson (1972, p. 54) observed that the subdivisions of Lampyridae in use at the time, ''though of some practical

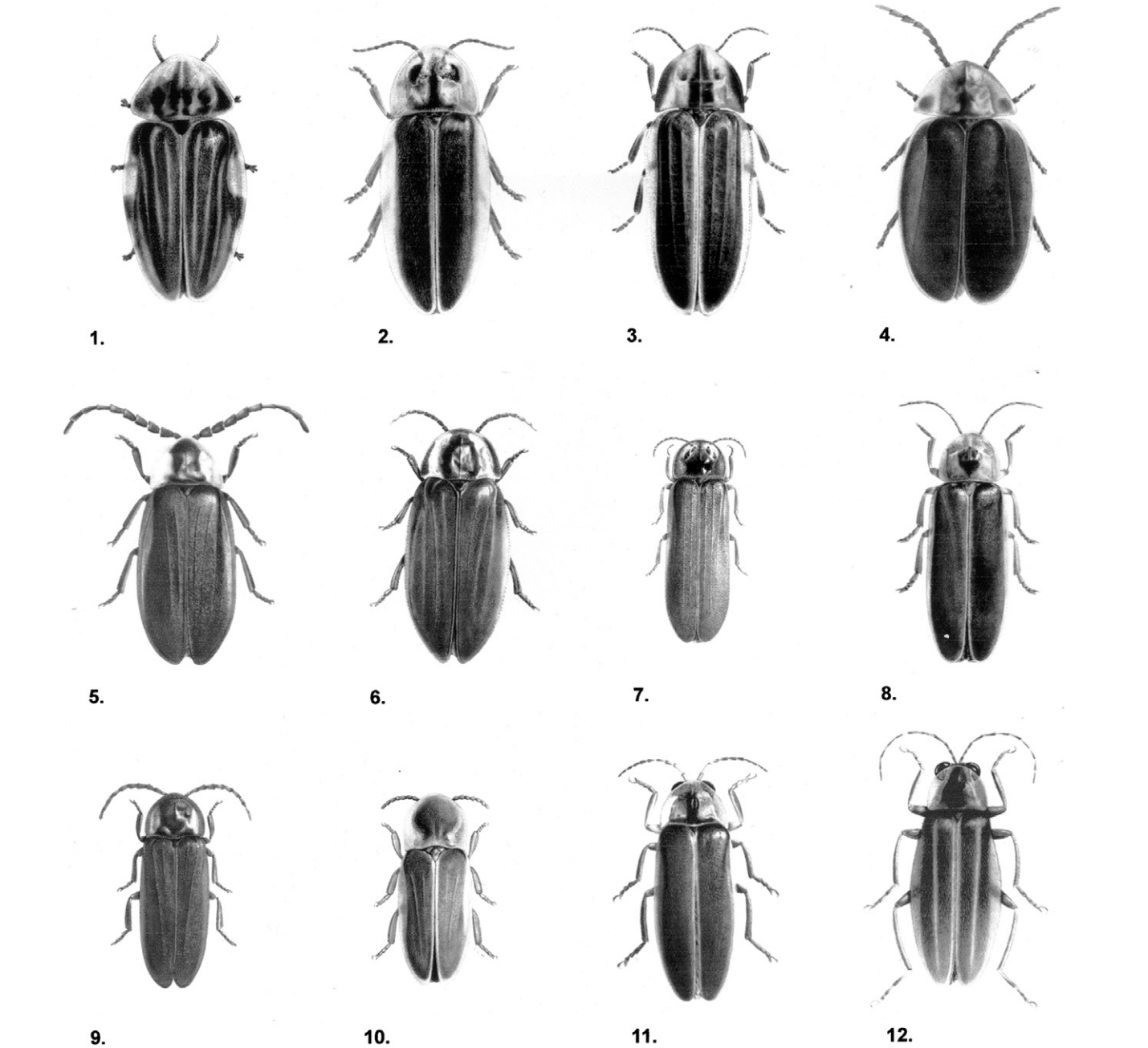

Fig. 1. North American firefly genera: 1, *Aspisoma*; 2, *Micronaspis*; 3, *Pyractomena*; 4, *Tenaspis*; 5, *Lucidota*; 6, *Ellychnia*; 7, *Phausis*; 8, *Photinus*; 9, *Pyropyga*; 10, *Pleotomodes*; 11, *Bicellonycha*; 12, *Photuris*. Carbon dust drawings by Laura Line.

Please cite this article in press as: Stanger-Hall, K.F. et al., Phylogeny of North American fireflies (Coleoptera: ..., Mol. Phylogenet. Evol. (2007), doi:10.1016/j.ympev.2007.05.013

utility, are probably not very natural''. The current classification of lampyrids into subfamilies is based on overall similarities such as the degree of retractability of the head under the head shield (pronotum), the number of visible ventral abdominal segments, the position of adult light organs (if present), and the size and shape of mouthparts and face shields (e.g. McDermott, 1964; Crowson, 1972). Many lampyrid genera, species groups, and species are differentiated by details that occur in male aedeagi (Green, 1956, 1957, 1959, 1961), but in some instances, and in *Photuris* in particular, male flash patterns, signals that are used during mate search, are necessary to identify species (Barber, 1951; Lloyd, 1969, 2002). These two character domains are intimately connected with sexual selection and mate choice.

1.2. Evolution of sexual signals in lampyrids

All lampyrids produce light at some stage in their life cycle, i.e. all known lampyrid larvae produce a faint glow using a paired larval light organ on the eighth abdominal segment (Branham and Wenzel, 2003). Based on morphological data, bioluminescence evolved early in the evolution of the cantharoid beetles, and lampyrids seem to have retained their larval bioluminescence from an early cantharoid ancestor (Branham and Wenzel, 2001, 2003). In contrast, adult lampyrids vary greatly in the presence, location, shape and use of adult light organs (Branham and Wenzel, 2003). As a consequence, only some lampyrids produce light as adults, whereas others mainly use chemical signals for mate attraction (Lloyd, 1997; Branham and Wenzel, 2003).

Lloyd (1997) distinguished three dominant signal modes among the mate attraction signals of the approximately 120 described North American (NA) species: (1) Chemical signals (pheromones): ''dark fireflies'' (e.g. *Ellychnia, Pyropyga, Lucidota*) produce no light as adults and are active during the day; they release chemical signals to attract mates. (2) Glows (continuous light signals): ''glowworm fireflies'' (e.g. *Microphotus, Phausis, Pleotomodes*) tend to have larvae-like females who spend the day in underground burrows and emerge at night, emitting a continuous glow. This glow (short distance) in combination with pheromones (long distance) attracts males who will fly towards the glow, but usually do not signal themselves. (3) Flashes (short intermittent light signals): ''lightningbug fireflies'' (e.g. *Photinus, Photuris, Pyractomena*) are the most commonly observed. They are active at dusk or in the dark and both males and females use species-specific light signals to communicate with each other in an interactive visual morse-code that identifies the species and the sex of the signaler. Some genera (e.g. *Pleotomus)* and individual species within genera (e.g. *Phausis reticulata)* may represent intermediate stages in signal evolution (e.g. *Pleotomus* males glow when disturbed). In addition, Ohba (1983, 2004) proposed two additional categories for Japanese lampyrids: (4) pheromones as the main signal mode, accompanied by a weak glow that is emitted during daytime or early dusk (e.g. *Cyphonocerus*), and (5) a long flash (e.g. *Luciola cruciata*). For the purpose of this study we focused on the evolution of the first four signal modes (pooling long and short flashes). We were especially interested in whether the ancestral signal mode in adult fireflies was indeed pheromones as suggested by morphology (e.g. McDermott, 1964; Branham and Wenzel, 2003), and if yes, then how light signaling may have evolved in adult lampyrids.

With this study we document the phylogenetic relationships of 16 North American lampyrid genera and the related genus *Pterotus* based on nuclear 18S and mitochondrial 16S and COI sequence data. We chose the NA lampyrids as a starting point, because they were the most accessible to us, and with 16 genera provide a diverse, but not unmanageable number of taxa for analysis. This is the first attempt to elucidate the phylogenetic relationships of the NA lampyrid fauna, which (like the faunas of Europe and Asia), seems to be the result of several invasions (McDermott, 1964). To test McDermott's suggestion, we incorporated all available sequence data of lampyrid genera from Europe, Asia and tropical America into an extended phylogenetic analysis. Aided by this phylogeny, we retrace the evolution of four sexual signal modes (pheromones only, pheromones and weak light glows, light glows, and light flashes) during the history of this group. In addition, the presented phylogeny of the North American lampyrids will provide a new framework with which to examine and compare the previously used and often conflicting data that until now were the only available attempts to classify these lampyrids. Once a sound phylogeny of the Lampyridae is established, we will not only be able to study the evolution of sexual signals, but also the many ecological specializations in this group.

2. Materials and methods

Lampyrids belonging to 16 different genera were collected for this study from across the United States and identified to species by J.E.L. These 16 genera included all 15 NA genera, and the genus *Aspisoma*, occurring as a rare migrant from Central America (*Tenaspis*, seemingly an occasional migrant from Mexico, was not collected and thus not included in the present analysis). The genus *Pterotus* (of uncertain family status: Branham and Wenzel, 2001) was included. In addition, we obtained six lampyrid species (from six genera) from Europe and Asia. These specimens were collected and identified by R. De Cock (*Lampyris noctiluca, Phosphaenus hemipterus* and *Lamprohiza splendidula*) and M.L. Wang (*Diaphanes formosus, Lychnuris formosana* and *Luciola* sp.). Net-winged beetles (Lycidae), identified as the sister group of lampyrids in another analysis (Stanger-Hall and Cicero, in preparation), were used as an outgroup (non-lampyrids) for this study, as well as one representative of the phengodids and the cantharids that have been proposed to have close relationships with individual firefly taxa in the past (e.g. Crowson, 1972; Branham

Please cite this article in press as: Stanger-Hall, K.F. et al., Phylogeny of North American fireflies (Coleoptera: ..., Mol. Phylogenet. Evol. (2007), doi:10.1016/j.ympev.2007.05.013

and Wenzel, 2001). Lycids were collected and identified by Joe Cicero.

2.1. DNA extraction and amplification

Specimens were stored in 95% ethanol at 4 °C. Between one and three legs (depending on the size of the individual) were removed from the specimen for DNA extraction, the rest of the body was preserved as a voucher specimen (in 95% ethanol at −20 °C). DNA was extracted using a Chelex extraction (Biolabs) or a Qiagen DNAeasy extraction kit. Portions of two mitochondrial (16S, COI) and one nuclear (18S) gene were amplified using the polymerase chain reaction (for PCR primers see Table 1). In both cases the initial denaturation was 94 °C for 2–4 min. Amplification conditions for the mitochondrial genes were 35 cycles of 94 °C for 60 s, 44 °C for 45–90 s, and 72 °C for 60–90 s. For the nuclear gene we used 35 cycles of 94 °C for 60 s, 50 °C for 60 s, and 72 °C for 60 s. The final extension was 5–7 min at 72 °C. Sequencing reactions (25 cycles) were run at 94 °C for 10 s, 50 °C for 5 sec, and 60 °C for 4 min. The sequences were analyzed in both directions using an ABI 3100 capillary sequencer. The resulting DNA sequences for each individual were aligned separately for each gene segment using Seqman (DNASTAR Inc., Madison, Wisconsin). The resulting consensus sequences were imported into MacClade (Maddison and Maddison, 2000) for final alignment by hand (sequences are available from Genbank; Table 2). After excluding an ambiguous AT-rich segment of 34 basepairs (bp) and several shorter segments from the 16S alignment (corresponding to bp 14443–14445, 14491–14524, 14595, 14596, and 14705–14707 of the 16S gene in *Pyrocoelia*: Genbank AF 452048), the alignment included more than 3400 bp. However, due to stretches of missing data in individual taxa (due to differences in primer binding and sequencing success) and the possibility that these unduly influence the phylogenetic analysis (Lemmon et al. unpublished data), the final alignment was reduced to 1906 bp. Missing data due to actual sequence length variation (exclusively observed within *Pyractomena*) were included in the analysis. To the final alignment of 1906 bp individual species contributed 211–500 bp of 18S sequence (corresponding to bp 785–944 and 970–1305 in *Callopteron*; Genbank AF 423764), 270–320 bp of 16S sequence (corresponding to bp 14371–14490 and 14525–14731 in *Pyrocoelia*; Genbank AF 452048), and 1003–1060 bp of COI sequence (corresponding to bp 3377–3812 and 3885–4508 in *Pyrocoelia*; Genbank AF 452048).

Pairwise ILD tests (Farris et al., 1995) as implemented in PAUP (Swofford, 2002) were conducted for all three data partitions (one for each gene) after removal of invariable characters. Two pairwise tests (18S-COI: $p = 0.17$ and 16S-COI: $p = 0.70$) were above the p-value suggested by Cunningham (1997) as a criterion for combinability, one test (18S–16S: $p = 0.008$) was below. It is assumed that by combining partitions with p-values above 0.01 the accuracy of the resulting phylogeny may be improved, but will not be reduced, and that by combining partitions with p-values below 0.001 the results will suffer (Cunningham, 1997). Since the ILD test for all pairwise tests was above or close to the suggested threshold for combinability, and the p-value for all three data sets together was $p = 0.074$, we combined all individual gene sequences into one large concatenated data set (ranging from 1533 to 1880 bp per species). In addition, Genbank sequences were available for 27 lampyrid species (10 genera) from Japan and Korea, contributing 319–320 bp of 16S sequence per species to the final data set. Three of these species contributed an additional 403–417 bp of COI sequence (see Table 2).

Table 1
PCR primers used in this study (letter designations of mitochondrial primers follow Simon et al., 1994)

Gene	Primer ID	Primer sequence (5′ to 3′)	References
18S	18Sai[a]	CCTGAGAAACGGCTACCACATC	Whiting et al. (1997)
	18Sbi[a]	GAGTCTCGTTCGTTATCGGA	Whiting et al. (1997)
16S	LR-J-12887[a] (16sbr)	CCGGTCTGAACTCAGATCACGT	Vogler and DeSalle (1993) Simon et al. (1994)
	LR-J∼13020[a] (16S401)	ACGCTGTTATCCCCAAGGTA	This study
	LR-N∼13374[a] (16S041)	TAAGGTCTAATCTCAATGA	This study
	LR-N-13398[a] (16sar)	CGCCTGTTTAACAAAAACAT	Vogler and DeSalle (1993) Simon et al. (1994)
	LR-J-13375 (16sc)	TCAGTGAGCAGGTTAGAC	Simon et al. (1994)
COI	C1-J∼1500[a] (LCO)	GGTCAACAAATCATAAAGATATTGG	Baldwin et al. (1996)
	C1-N∼2150[a] (HCO)	TAAACTTCAGGGTGACCAAAAAATCA	Baldwin et al. (1996)
	C1-J-1718[a]	GGAGGATTTGGAAATTGATTAGTTCC	Simon et al. (1994)
	C1-J-1718m	GGAGGCTTCGGAAATTGATTAGTTCC	This study
	C1-J-1751	GGATCACCTGATATAGCATTCCC	Simon et al. (1994)
	C1-J-1751ff	GGGGCTCCTGATATAGCTTTTCC	This study
	C1-J-2183[a]	CAACATTTATTTTGATTTTTTGG	Simon et al. (1994)
	C1-N-2191[a]	CCCGGTAAAATTAAAATATAAACTTC	Simon et al. (1994)
	C1-J-2441	CCAACAGGAATTAAAATTTTTAGATGATTAGC	Simon et al. (1994)
	TL2-N-3014[a]	TCCAATGCACTAATCTGCCATATTA	Simon et al. (1994)

[a] Most commonly used primers (the other primers were used for individual taxa that did not amplify with common primers).

Please cite this article in press as: Stanger-Hall, K.F. et al., Phylogeny of North American fireflies (Coleoptera: ..., Mol. Phylogenet. Evol. (2007), doi:10.1016/j.ympev.2007.05.013

Table 2
Worldwide taxa used in the present analysis and their Genbank accession numbers

Family	Subfamily	Genus	Species	Origin	GB 18S	GB 16S	GB COI
CANTHARIDAE					EU009213	EU009250	EU009287
LYCIDAE		Caenia	amplicornis (LeConte 1881)	NA[a]	EU009215	EU009252	EU009289
LYCIDAE		Lycus	fernandezi (Duges 1878)	NA	EU009214	EU009251	EU009288
PHENGODIDAE					EU009212	EU009249	EU009286
LAMPYRIDAE	Cyphonocerinae	Cyphonocerus	ruficollis (Kiesenwetter 1879)	Japan		AB009926[b]	
LAMPYRIDAE	Cyphonocerinae	Pollaclassis	bifaria (Say 1835)	NA	EU009221	EU009258	EU009295
LAMPYRIDAE	Lampyrinae	Aspisoma	species	Panama	EU009248	EU009285	EU009322
LAMPYRIDAE	Lampyrinae	Diaphanes	formosus (Olivier 1910)	Taiwan	EU009243	EU009280	EU009317
LAMPYRIDAE	Lampyrinae	Ellychnia	californica (Motschulsky 1853)	NA	EU009218	EU009255	EU009292
LAMPYRIDAE	Lampyrinae	Ellychnia	corrusca (Linnaeus 1767) complex	NA	EU009225	EU009262	EU009299
LAMPYRIDAE	Lampyrinae	Lamprohiza	splendidula (Linnaeus 1767)	Belgium	EU009245	EU009282	EU009319
LAMPYRIDAE	Lampyrinae	Lampyris	noctiluca (Linnaeus 1767)	Europe	EU009247	EU009284	EU009321
LAMPYRIDAE	Lampyrinae	Lucidina	accensa (Gorham 1883)	Japan		AB009923[b]	
LAMPYRIDAE	Lampyrinae	Lucidina	biplagiata (Motschulsky 1866)	Japan		AB009922[b]	
LAMPYRIDAE	Lampyrinae	Lucidina	okadai (Nakane et Ohbayashi 1949)	Japan		AB009924[b]	
LAMPYRIDAE	Lampyrinae	Lucidota	atra (Olivier 1790)	NA	EU009219	EU009256	EU009293
LAMPYRIDAE	Lampyrinae	Lychnuris	formosana (Olivier 1911)	Taiwan	EU009242	EU009279	EU009316
LAMPYRIDAE	Lampyrinae	Micronaspis	floridana (Green 1948)	NA	EU009240	EU009277	EU009314
LAMPYRIDAE	Lampyrinae	Microphotus	angustus (LeConte 1874)	NA	EU009227	EU009264	EU009301
LAMPYRIDAE	Lampyrinae	Paraphausis	eximia (Green 1949)	NA	EU009223	EU009260	EU009297
LAMPYRIDAE	Lampyrinae	Phausis	reticulata (Say 1825)	NA	EU009237	EU009274	EU009311
LAMPYRIDAE	Lampyrinae	Phosphaenus	hemipterus (Fourcroy 1785)	Belgium	EU009246	EU009283	EU009320
LAMPYRIDAE	Lampyrinae	Photinus	australis (Green 1956)	NA	EU009224	EU009261	EU009298
LAMPYRIDAE	Lampyrinae	Photinus	floridanus (Fall 1927)	NA	EU009232	EU009269	EU009306
LAMPYRIDAE	Lampyrinae	Photinus	punctulatus (LeConte 1851)	NA	EU009238	EU009275	EU009312
LAMPYRIDAE	Lampyrinae	Photinus	pyralis (Linnaeus 1767)	NA	EU009239	EU009276	EU009313
LAMPYRIDAE	Lampyrinae	Photinus	tanytoxis (Lloyd 1966)	NA	EU009241	EU009278	EU009315
LAMPYRIDAE	Lampyrinae	Pleotomodes	needhami (Green 1948)	NA	EU009231	EU009268	EU009305
LAMPYRIDAE	Lampyrinae	Pleotomus	pallens (LeConte 1866)	NA	EU009217	EU009254	EU009291
LAMPYRIDAE	Lampyrinae	Pristolycus	sagulatus (Gorham 1883)	Japan		AB009925[b]	
LAMPYRIDAE	Lampyrinae	Pyractomena	angulata (Say 1825)	NA	EU009233	EU009270	EU009307
LAMPYRIDAE	Lampyrinae	Pyractomena	borealis (Randall 1838)	NA	EU009222	EU009259	EU009296
LAMPYRIDAE	Lampyrinae	Pyractomena	palustris (Green 1958)	NA	EU009235	EU009272	EU009309
LAMPYRIDAE	Lampyrinae	Pyrocoelia	atripennis (Lewis 1896)	Japan		AB009915[b]	
LAMPYRIDAE	Lampyrinae	Pyrocoelia	discicollis (Kiesenwetter 1874)	Japan		AB009916[b]	
LAMPYRIDAE	Lampyrinae	Pyrocoelia	fumosa (Gorham 1883)	Japan		AB009917[b]	
LAMPYRIDAE	Lampyrinae	Pyrocoelia	m. matsumurai (Nakane 1963)	Japan		AB009919[b]	
LAMPYRIDAE	Lampyrinae	Pyrocoelia	miyako (Nakane 1981)	Japan		AB009914[b]	
LAMPYRIDAE	Lampyrinae	Pyrocoelia	rufa (E. Olivier 1886)	Japan		AB009913[b]	
LAMPYRIDAE	Lampyrinae	Pyrocoelia	m. kumejimensis (Chujo et M.Sato 1972)	Japan		AB009920[b]	
LAMPYRIDAE	Lampyrinae	Pyrocoelia	oshimana (Nakane 1985)	Japan		AB009918[b]	
LAMPYRIDAE	Lampyrinae	Pyropyga	nigricans (Say 1823)	NA	EU009220	EU009257	EU009294
LAMPYRIDAE	Lampyrinae	Pyropyga	decipiens (Harris 1836)	NA	EU009226	EU009263	EU009300
LAMPYRIDAE	Luciolinae	Curtos	costipennis (Gorham 1880)	Japan		AB009912[b]	
LAMPYRIDAE	Luciolinae	Curtos	okinawana (Matsamura 1918)	Japan		AB009911[b]	
LAMPYRIDAE	Luciolinae	Hotaria	papariensis (Doi 1932)	Korea		AF272696[d]	
LAMPYRIDAE	Luciolinae	Hotaria	parvula (Kiesenwetter 1874)	Japan		AB009909[b]	AF485364[e]
LAMPYRIDAE	Luciolinae	Hotaria	tsushimana (Nakane 1970)	Japan		AB009910[b]	
LAMPYRIDAE	Luciolinae	Luciola	cruciata (Motschulsky 1854)	Japan		AB009904[b]	AF360953[c]
LAMPYRIDAE	Luciolinae	Luciola	kuroiwae (Matsamura 1918)	Japan		AB009907[b]	
LAMPYRIDAE	Luciolinae	Luciola	lateralis (Motschulsky 1860)	Japan		AB009906[b]	AF360873[c]
LAMPYRIDAE	Luciolinae	Luciola	owadai (M.Sato et M.Kimura 1994)	Japan		AB009905[b]	
LAMPYRIDAE	Luciolinae	Luciola	species	Taiwan	EU009244	EU009281	EU009318
LAMPYRIDAE	Luciolinae	Luciola	yayeyamana (Matsamura 1918)	Japan		AB009908[b]	
LAMPYRIDAE	Ototretinae	Brachylampis	blaisdelli (Van Dyke 1939)	NA	EU009230	EU009267	EU009304
LAMPYRIDAE	Ototretinae	Drilaster	axillaris (Kiesenwetter 1879)	Japan		AB009927[b]	
LAMPYRIDAE	Ototretinae	Drilaster	kume-jima island	Japan		AB009928[b]	
LAMPYRIDAE	Ototretinae	Stenocladius	flavipennis (Kawashima 1999)	Japan		AB009930[b]	
LAMPYRIDAE	Ototretinae	Stenocladius	shirakii (Nakane 1981)	Japan		AB009929[b]	
LAMPYRIDAE	Photurinae	Bicellonycha	wickershamorum (Cicero 1982)	NA	EU009228	EU009265	EU009302
LAMPYRIDAE	Photurinae	Photuris	lucicrescens (Barber 1951) group	NA	EU009216	EU009253	EU009290
LAMPYRIDAE	Photurinae	Photuris	quadrifulgens (Barber 1951)	NA	EU009236	EU009273	EU009310
LAMPYRIDAE	Photurinae	Photuris	tremulans (Barber 1951)	NA	EU009234	EU009271	EU009308

(*continued on next page*)

Table 2 (*continued*)

Family	Subfamily	Genus	Species	Origin	GB 18S	GB 16S	GB COI
Elateroidae incertae sedis		Pterotus	obscuripennis (LeConte 1859)	NA	EU009229	EU009266	EU009303
RHAGOPHTHALMIDAE		Rhagophtalmus	ohbai (Wittmer 1994)	Japan		AB009931[b]	

The current classification of North American firefly genera (Lampyridae) is based on McDermott (1964) and modified by Branham and Wenzel (2003). [a]NA, North America; Genbank (GB) data: [b]Suzuki (1997);[c]Kim et al. (2001a); [d]Kim et al. (2001b); [e]Choi et al. (2003). An alignment is available on TreeBASE (http://www.treebase.org/treebase/index.html).

2.2. Data analysis

Two different data sets were analyzed: a North American data set and a worldwide data set (including the NA taxa). The NA data set included 31 taxa (five non-lampyrids and 26 lampyrids): two lycid species (from two genera), one phengodid species, one cantharid species, one *Pterotus* species (a genus with unclear family status), and 26 lampyrid species (from 16 genera). Each of these taxa was represented by sequence data from all three genes (COI, 16S, 18S). The worldwide dataset included the complete NA data set, three European and three Asian species that also included sequence data from all three gene regions, and additional sequences available from Genbank (mostly 16S and a few COI sequences). Altogether the worldwide data set included 65 taxa: the same five non-lampyrids as the NA analysis, *Rhagophthalmus ohbai*, previously classified as a lampyrid (subfamily Rhagophthalminae: e.g. McDermott, 1964), but then established as a separate family, Rhagophthalmidae (Wittmer and Ohba, 1994), and 59 lampyrid species (from 30 genera).

2.3. Phylogenetic methods

Both data sets (NA and World) were analyzed individually with a maximum likelihood analysis (using a single evolutionary model for the entire data set) and with a Bayesian analysis (using a different model for each data partition). In both analyses the phengodid specimen was designated as the outgroup taxon to root the trees.

2.3.1. Maximum likelihood analysis

The maximum likelihood analyses were performed using the successive approximation approach to parameter optimization (Swofford et al., 1996), which has recently been shown to be as accurate as the full optimization of parameters in the ML estimation of tree topology (Sullivan et al., 2005). Starting with a parsimony analysis (stepwise addition, random addition sequence, $n = 100$ replicates), a set of most parsimonious trees was generated. Likelihood scores were estimated for all these individual trees using a predetermined model of evolution. The appropriate model of evolution for the ML analyses was determined in Modeltest (LRT and AIC, v 3.7, Posada and Crandall, 1998; the AIC was given priority when the two tests favored two different models: Posada and Buckley, 2004). The parsimony tree with the best likelihood score was selected, and its estimated model parameters were used for a subsequent ML analysis in PAUP (Swofford, 2002). The estimated model parameters of the resulting ML tree were in turn submitted as new model parameters for the next ML analysis, and so on, until subsequent ML analyses yielded the same tree and model parameters as the previous analysis. This final tree provided the best ML hypothesis for that data set.

2.3.2. Bayesian analysis

Bayesian analyses were conducted in MrBayes version 3 using different models of evolution for different data partitions (Ronquist and Huelsenbeck, 2003). Modeltest (v 3.7, Posada and Crandall, 1998) suggested GTR + G + I as the most appropriate for both mitochondrial (16S + COI) data partitions; for the nuclear data partition (18S), the TrN + I + G model (a submodel of the GTR + G + I model with one transversion and two transition classes) was selected. However, since the TrN + I + G model is not implemented in MrBayes, we chose the slightly more complex GTR + G + I model instead (based on Huelsenbeck and Rannala, 2004, who showed that overparameterization, in contrast to underparameterization, does not lead to any bias in the resulting posterior probabilities). To identify the most appropriate number of partitions for our analyses, we ran three Bayesian analyses: (1) using a single model (GTR + G + I) of evolution applied to all three genes, (2) using two model partitions of the GTR + G + I model (one for ribosomal and one for protein-coding genes), and (3) using three model partitions of the GTR + G + I model (one for each gene). The three-model-partition analysis (allowing estimation of the model parameters independently for each gene) returned considerably higher Bayes factors (the ratio of the marginal likelihoods under two models: Huelsenbeck et al., 2002) and was therefore chosen as the most appropriate model for our analysis. The priors were equiprobable on topologies and the defaults were used for the remaining parameters (MrBayes v.3, Ronquist and Huelsenbeck, 2003).

For each analysis (NA and World) we ran four different MrBayes runs of 5 million generations each. Within each run we used four MCMC (Markov Chain Monte Carlo) chains with a default incremental heating parameter of 0.2. Subsequently, we entered the Bayesian runs into MrConverge v1.0b1 (A. Lemmon, unpublished), to determine the burn-in phase (the number of generations before

apparent stationarity), and to compare the results of our four independent runs to check for convergence and to ensure that the chains were providing valid samples from the posterior probability distribution. After convergence following the initial burn-in phase was confirmed, the samples from the stationary phases of the four independent runs were pooled (e.g. Nylander et al., 2004), and a Bayesian consensus tree with posterior probabilities for individual branches was computed.

The results of our phylogenetic analyses are presented as ML trees. Our conclusions regarding the support for individual branches on these trees are based on posterior probabilities from our Bayesian analyses (allowing optimization of the GTR + I + G model separately for each gene). The results of NA and World analyses were compared to assess the effect of taxon sampling on the phylogenetic relationships of the North American lampyrids, and to identify multiple origins of the NA lampyrid fauna.

We assessed the support for published and alternative grouping hypotheses of taxa in our data set, by analyzing the post-burn in samples of our worldwide Bayesian analysis. A Bayesian analysis is designed to analyze support for individual groupings within the data set irrespective of other groupings (returns marginal probabilities). This makes it an excellent tool to analyze the support for alternative grouping hypotheses within a tree. Different grouping hypotheses were formulated as alternative trees in MacClade (Maddison and Maddison, 2000) and loaded as constraint trees into PAUP (Swofford, 2002). Using each constraint tree as a filter, we obtained estimates of the posterior probability of each grouping hypothesis as the proportion of all posterior samples (post-burn in) compatible with that hypothesis.

2.4. Evolution of light signals

Information on signals used during mate search was obtained from the literature and classified into one of four categories (following Lloyd, 1997 and Ohba, 2004): (1) pheromones: no light production possible (due to lack of light organs) or observed; (2) pheromones and weak glows: pheromones are the main signal during mate search, but weak glows are produced as well. Species in this category tend to be diurnal just like species that use pheromones exclusively. (3) Continuous (long) glows, which are emitted exclusively by females in many species, but also by males in others; and (4) short flashes, which are usually emitted by both males and females in reciprocal signaling during mate search. In the first two categories, pheromones are the major mating signal, but in the latter two, light is used as the main sexual signal, and these species tend to be nocturnal. These four sexual signal modes were mapped onto the molecular phylogeny to identify potential changes in sexual signal mode during the evolutionary history of lampyrids, and to generate testable hypothesis on the evolution of sexual signal modes in this group.

3. Results

3.1. Phylogenetic relationships of the North American lampyrids

The ML tree of our NA analysis had a ln likelihood score of −16621.00917. Modeltest yielded the most complex model (GTR + G + I) as the most appropriate for this analysis. The GTR + G + I model was based on an estimated proportion of invariable sites (P-inv) of 47.2% and an estimated gamma parameter (G shape) value of 0.629550. The base compositions were AT-biased: A, 34.4%; C, 14.24%; G, 15.78%; T, 35.54%. The ML tree (Fig. 2) is shown with Bayesian posterior probabilities (PP) that were estimated from a (post-burn in) sample of 199,240 Bayesian trees. Based on the Bayesian analysis, the family Lampyridae, including *Pterotus*, is a monophyletic group (PP = 1.0) with *Pterotus* (Elateroidea *incertae sedis*) clearly nesting within this family (Fig. 2). The more basal genera within NA are *Pterotus* (Elateroidea *incertae sedis*), *Pollaclassis* (Cyphonocerinae), and *Brachylampis* (Ototretinae), who form a sistergroup (PP = .91) to all remaining NA fireflies, and *Phausis* (Lampyrinae), whose exact position remains unresolved (PP < .5; Fig. 2).

The remaining NA lampyrids form one clade (PP = 1.0) with two strongly supported subclades (PP = 1.0): the Photurinae (*Bicellonycha* and *Photuris*) and the NA Lampyrinae (except *Phausis*). Within NA the subfamily Lampyrinae is represented by four tribes: Cratomorphini, Lampyrini, Photinini and Pleotomini. The tribes Pleotomini and Lampyrini are both monophyletic (PP = 1.0), and form a sistergroup (PP = .98) in a clade (PP = .93) with *Aspisoma* (Cratomorphini). The tribes Cratomorphini and Photinini are polyphyletic (Fig. 2). The tribe Cratomorphini is represented by at least two separate groups: *Aspisoma* and *Micronaspis* with *Pyractomena* (their exact relationship remains unresolved, PP < .5). The tribe Photinini is split into three separate groups: the genus *Phausis*, the genus *Lucidota*, and a *Pyropyga–Ellychnia–Photinus* clade (PP = .89; Fig. 2). In contrast to all other NA genera, the genus *Photinus* is not monophyletic, but contains the genus *Ellychnia* (PP = 1.0).

3.2. Worldwide phylogenetic relationships

The ML tree of the worldwide analysis had a ln likelihood score of −20736.55475. Modeltest yielded the most complex model (GTR + G + I) as the most appropriate for this analysis. It was based on an estimated proportion of invariable sites (P-inv) of 45.4% and an estimated gamma parameter (G shape) value of 0.5929. The base compositions were AT-biased: A, 36.79%; C, 12.6%; G, 15.07%; T, 35.51%. The ML tree (Fig. 3) is shown with Bayesian posterior probabilities (PP) that were estimated from a sample of 131,700 Bayesian trees. The lampyrids form a paraphyletic group (PP = .86) in the worldwide analysis, with *Rhagophthalmus* (Rhagophthalmidae)

Please cite this article in press as: Stanger-Hall, K.F. et al., Phylogeny of North American fireflies (Coleoptera: ..., Mol. Phylogenet. Evol. (2007), doi:10.1016/j.ympev.2007.05.013

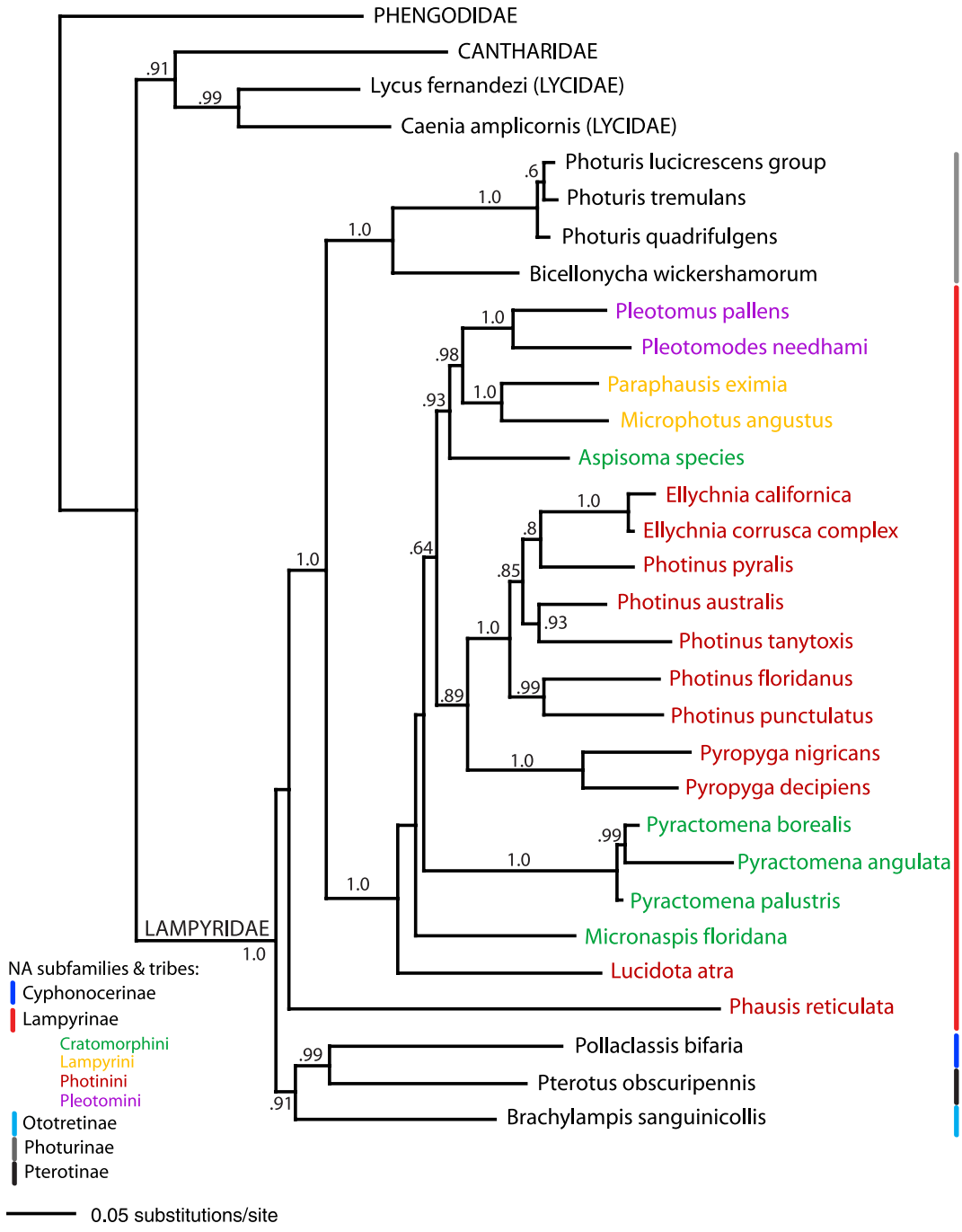

Fig. 2. The phylogenetic relationships of North American firefly genera and their current classification into subfamilies and tribes (within Lampyrinae). Maximum likelihood tree with Bayesian posterior probabilities (based on a consensus of 199,240 trees). Due to space limitations, posterior probabilities (PP) are either given above, below or to the right of the respective branches.

nesting within lampyrids in 99.99% of trees (it grouped with the OG in 10 of 131,700 trees). *Stenocladius* (*Ototretinae*) grouped within lampyrids in 84.8% ($n = 111{,}687$) of all trees, it grouped as a basal sister taxon to the remaining lampyrids in 0.6% ($n = 767$) of all trees, and it grouped with the OG (with phengodids, basal to cantharids and lycids) in 14% ($n = 18{,}806$) of all trees. The Bayesian analysis supported a monophyletic Cyphonocerinae (*Pollaclassis* and *Cyphonocerus*: PP = .75), and (deviating from the ML tree) grouped *Stenocladius* basal to Luciolinae and *Pristolycus* in an unresolved position with *Pterotus* and *Rhagophthalmus*, and *Pollaclassis* and *Cyphonococerus* (Fig. 3). In contrast, the monophyly of the Ototretinae (grouping *Stenocladius* with *Brachylampi* and *Drilaster*) was only supported by 0.14% ($n = 185$) of all Bayesian trees.

Geography is not a good predictor of phylogeny in lampyrids. Neither Asian, European, nor North American taxa form monophyletic groups (Fig. 3). Individual genera of NA lampyrids form sistergroup relationships with Asian

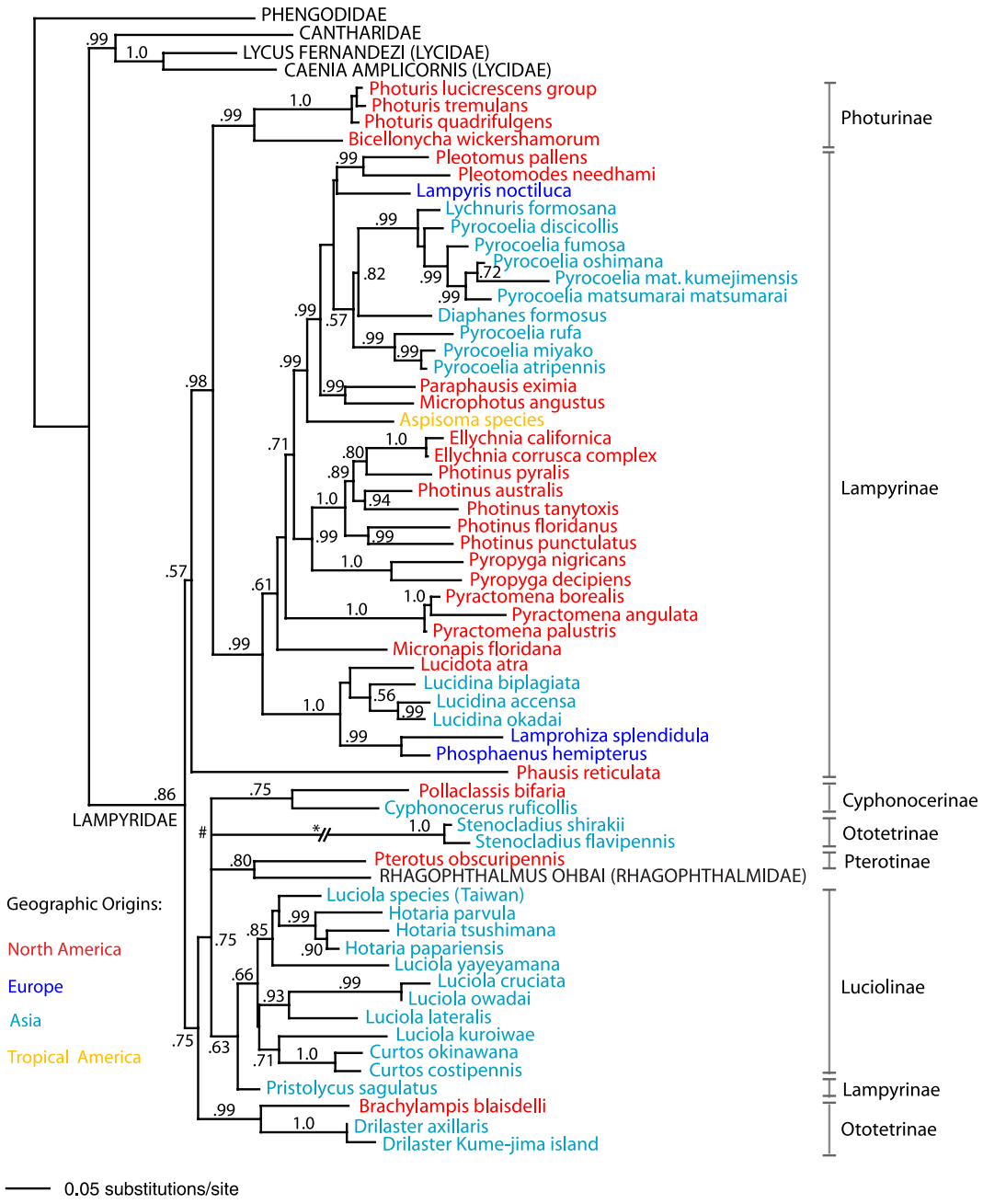

Fig. 3. The phylogenetic relationships of North American (red), European (blue), and Asian (green) firefly species, and fireflies from tropical America (orange). Maximum likelihood tree with Bayesian posterior probabilities (consensus of 131,696 trees). Their current classification into subfamilies is shown on the right. #, the ML tree grouped *Stenocladius* as a sister taxon to *Cyphonocerus*, but this relationship was unsupported by the Bayesian analysis. As a result we show *Stenocladius* in an unresolved position within a clade containing *Pollaclassis* and *Cyphonocerus*; *Pterotus* and *Rhagophthalmus*; and *Pristolycus* and *Luciola*; and *Hotaria* and *Curtos*. *, the branch leading to *Stenocladius* is twice the length shown.

and European lampyrids and lampyrids from tropical America (Central and South America). The NA genus *Brachylampis* (Ototretinae) groups with *Drilaster* (Ototretinae from Asia; PP = .99); *Pollaclassis* (Cyphonocerinae) groups with *Cyphonocerus* (Cyphonocerinae from Asia) and *Pterotus* (Elateroidea *incertae sedis*) groups with *Rhagophthalmus* (Rhagophthalmidae from Asia; PP = 0.8; Fig. 3). The NA genus *Lucidota*, the Asian genus *Lucidina*, and *Lamprohiza* and *Phosphaenus* from Europe form a monophyletic group (PP = 1.0). Furthermore, the genus *Aspisoma* from tropical America groups as a basal taxon (PP = .99) of a clade containing *Pleotomus, Pleotomodes, Paraphausis*, and *Microphotus* from NA, *Lampyris* from Europe, and *Pyrocoelia* and *Diaphanes* from Asia (Fig. 3).

The 59 recognized lampyrid species in the present study represent six subfamilies: Cyphonocerinae, Lampyrinae, Luciolinae, Ototretinae, Photurinae, and Pterotinae (Fig. 3). The subfamily Photurinae is the only strongly supported (PP = .99) subfamily in our study, two subfamilies: Cyphonocerinae (PP = .75) and Luciolinae (PP = .66)

received moderate or weak Bayesian support, one subfamily (Pterotinae) is represented by a single species, and the remaining two subfamilies represented here (Ototretinae and Lampyrinae) are polyphyletic: Ototretinae were split into two different subgroups, and the Lampyrinae were split into two or three subgroups (depending on the exact position of *Phausis*: Fig. 3). With the exception of *Pristolycus* and *Phausis*, the remaining Lampyrinae form a monophyletic group (PP = .99). Within this latter group there are several strongly supported clades: (1) *Lucidota*, *Lucidina*, *Phosphaenus* & *Lamprohiza* form the basal clade (PP = 1.0); (2) *Pyropyga*, *Photinus* and *Ellychnia* (PP = .99); (3) *Aspisoma*, *Microphotus* and *Paraphausis*, *Pleotomus* and *Pleotomodes*, *Lampyris, Lychnuris*, *Pyrocoelia*, and *Diaphanes* (PP = .99). Within this latter clade, the genus *Pyrocoelia* is split into two separate groups: *P. rufa, P. miyako*, and *P. atripennis* in one group (PP = .99), and the remaining *Pyrocoelia* species with *Diaphanes* (Taiwan)

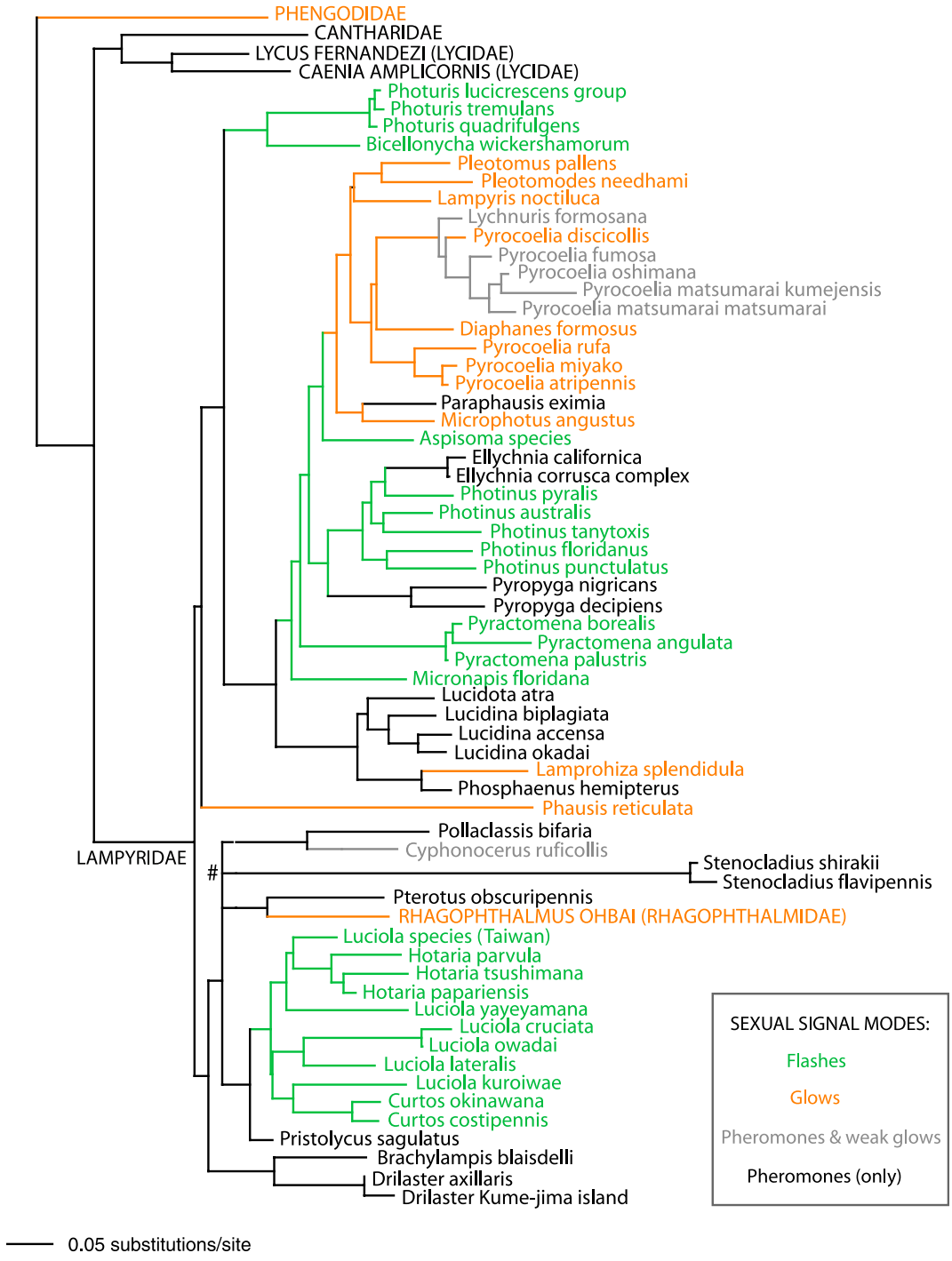

Fig. 4. Sexual signal modes in adult lampyrids (see Fig. 3 legend for analysis and branch support).

as the basal genus (PP = .82). The exact relationships of *Micronaspis* and *Pyractomena* with each other and with other lampyrine taxa remained unresolved (low PPs). The species in the subfamily Luciolinae that are represented here can be divided into at least three groups: (1) *L. lateralis, L. cruciata* and *L. owadai*, (PP = 0.93); (2) *L. yayemana*, *Luciola sp.* (Taiwan), and *Hotaria* (PP = 0.85); and (3) *L. kuroiwae* and *Curtos* (PP = 0.66).

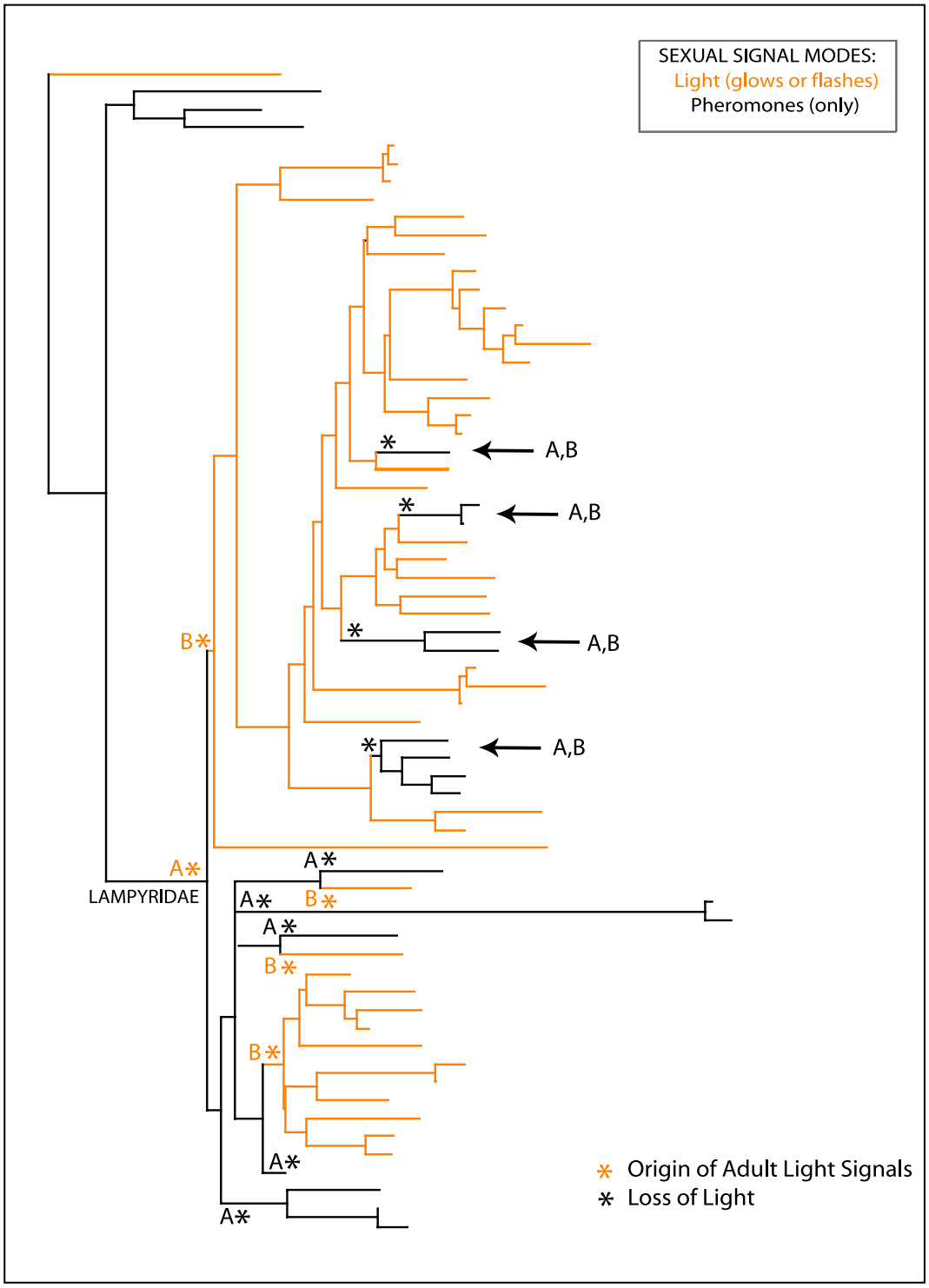

Fig. 5. Possible origins and losses of light as a sexual signal in lampyrids (see Fig. 3 legend for analysis and branch support). Scenario A, light signals originated once in ancestral adult lampyrids, and were subsequently lost nine times. Scenario B, ancestral lampyrids used pheromones as sexual signal, and the transition to sexual light signals evolved four times independently, followed by four losses. There are at least two other possible 10-step scenarios (multiple gains and losses), but neither is favored by any weighting where losses are considered as likely or more likely than gains. The color-coding of the branches reflects scenario B.

Please cite this article in press as: Stanger-Hall, K.F. et al., Phylogeny of North American fireflies (Coleoptera: ..., Mol. Phylogenet. Evol. (2007), doi:10.1016/j.ympev.2007.05.013

Increased taxon sampling and the missing data for Asian fireflies in the worldwide analysis had no direct effect on the resulting phylogenetic relationships of NA fireflies. The branching patterns stayed the same, only the support values (PP) changed.

3.3. Light signal evolution

Several relatively basal lampyrids (e.g. *Brachylampis, Drilaster*) in our molecular phylogeny use pheromones for mate attraction, however, another basal taxon (*Phausis*)

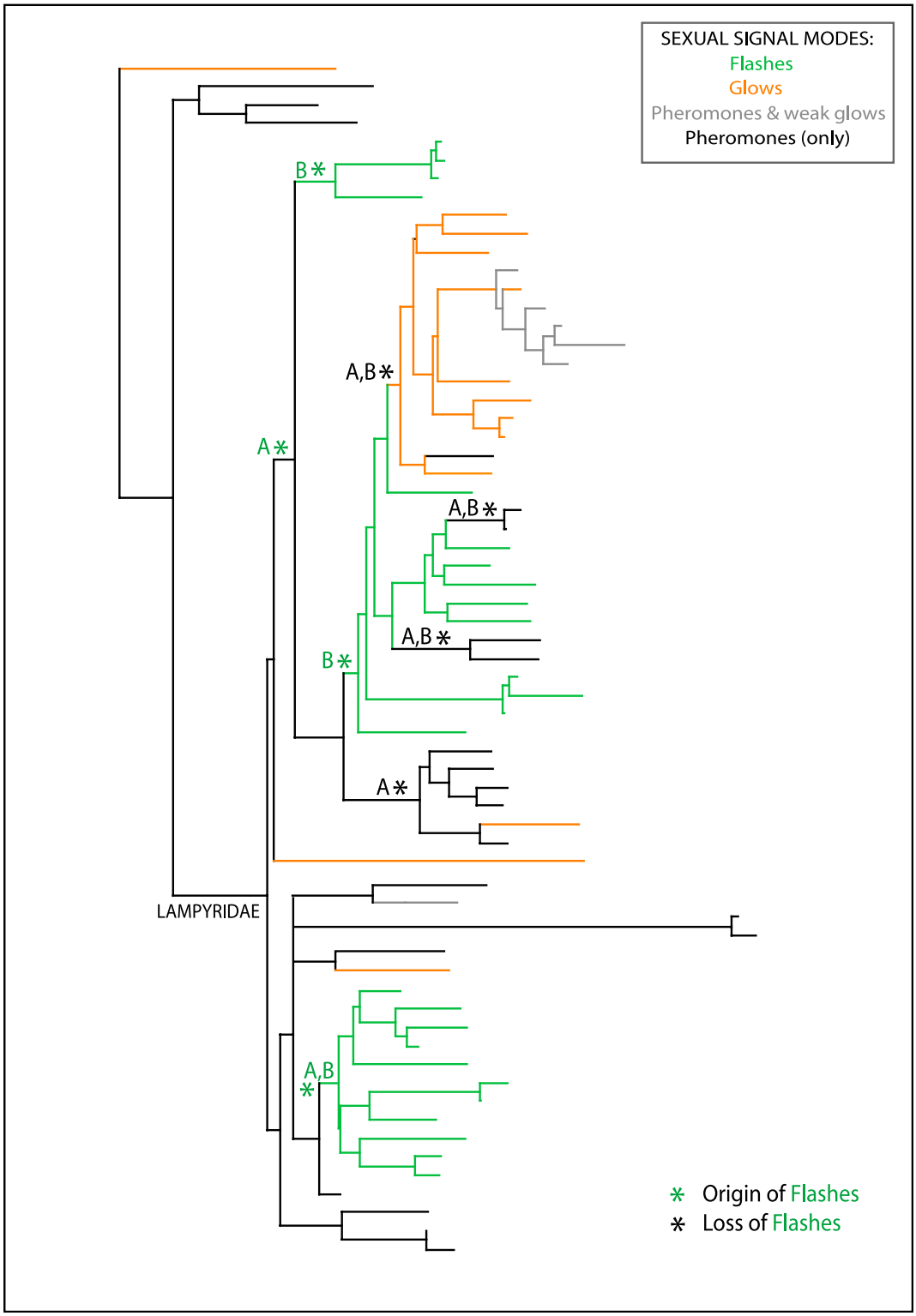

Fig. 6. Possible origins and losses of flashes as sexual signals in lampyrids (see Fig. 3 legend for analysis and branch support). Flashes originated twice (scenario A, followed by two losses) or three times (scenario B, followed by one loss) independently during the evolutionary history of the lampyrids in our study. Color-coding of branches reflects scenario B.

Please cite this article in press as: Stanger-Hall, K.F. et al., Phylogeny of North American fireflies (Coleoptera: ..., Mol. Phylogenet. Evol. (2007), doi:10.1016/j.ympev.2007.05.013

uses glows (Fig. 4). As a result, adult light signals may either have evolved in the ancestral lampyrid and subsequently been lost in *Brachylampis* and retained in *Phausis*, or the ancestral sexual signals of adult lampyrids may have been pheromones (retained by *Brachylampis*). Based on our analysis there are several possible scenarios for the evolution of adult light signals (Fig. 5). Scenario A requires only a single origin of sexual light signals (followed by nine losses, adding up to 10 changes overall). In contrast, scenario B requires up to four independent origins of adult light signals (followed by four losses, adding up to eight changes overall; Fig. 5). There are at least two more possible scenarios (not shown), but each would require at least 10 changes with multiple losses and gains. Overall scenario B requires the fewest number of changes. The use of flashes as sexual light signals originated at least twice (Fig. 6: scenario A, followed by four losses), and possibly three times (Fig. 6: scenario B, followed by three losses) independently. Short flashes were lost as sexual signals by being replaced by long glows (Fig. 6; $n = 1$) or by pheromones as the main sexual signal mode (Fig. 6: $n = 2$(B) or 3(A)).

4. Discussion

4.1. Phylogenetic relationships of NA lampyrids

Our molecular data support McDermott's suggestion (1964) that the NA lampyrid fauna did not originate from a single adaptive radiation, but is the result of several independent invasions instead. Our data also confirm that classification does not, for the most part, reflect phylogeny (McDermott, 1964), but more importantly, allow us to identify the exact conflicts that need to be resolved for classification and phylogeny to be compatible.

Pterotus is clearly a lampyrid and should be reestablished as a lampyrid taxon (e.g. subfamily Pterotinae: McDermott, 1964; Crowson, 1972; Lawrence and Newton, 1995). Similarly, based on molecular evidence, the status of *Rhagophthalmus* in a separate family (Wittmer and Ohba, 1994) should be reconsidered. In the past, *Rhagophthalmus* was classified as a member of the lampyrid subfamily Rhagophthalminae (McDermott, 1964). Subsequently the subfamily was transferred to the family Phengodidae by Crowson (1972), and eventually it gained family status, Rhagophthalmidae (Wittmer and Ohba, 1994). In our analysis it is closely linked (PP = .80) with *Pterotus* (Fig. 3). Our present analysis rules out that *Rhagophthalmus* or *Pterotus* belong in the outgroup with the other non-lampyrids (0.01% and 0% support, respectively), and clearly puts both within the Lampyridae (99.99% and 100%, respectively). In addition to Suzuki's 16S data (Suzuki, 1997), the inclusion of *Rhagophthalmus* within the Lampyridae is further supported by embryological evidence (Kobayashi et al., 2001). In contrast, the position of *Stenocladius* is more ambiguous. Although it grouped within lampyrids with a 86% support, the fact that it grouped within the outgroup in 14% of all Bayesian trees warrants a closer examination, especially since a recent morphological analysis also suggested a close affiliation of *Stenocladius* with phengodids (Branham and Wenzel, 2001). However, at present we cannot exclude the possibility that analysis artifacts (due to the large number of substitutions in the 16S gene responsible for the long branch leading to *Stenocladius)* such as long branch attraction (Felsenstein, 2004) may have led to the occasional grouping of *Stenocladius* with the outgroup taxa. In addition, it is possible that with increased sampling of basal taxa and with more sequence information from additional genes *Pterotus*, *Rhagophthalmus* and *Stenocladius* will move into a basal sister-group position to all remaining lampyrids. Therefore future studies are needed to reevaluate the status of these three taxa. Critical for this undertaking will be the inclusion of more lampyrids from tropical America (the presumed origin of Lampyrids: McDermott, 1964), Asia, and elsewhere (e.g. Africa and Australia).

Crowson (1972) considered the subfamily Ototretinae as a rather heterogeneous collection of genera. This is supported by our data. Only 0.14% of our posterior probability sample ($n = 185$ trees) included Ototretinae as a monophyletic group. Instead *Brachylampis* and *Drilaster* form a monophyletic group ($p = 0.99$), and in our Bayesian analysis *Stenocladius* groups in an unresolved position with Cyphonocerinae, Pterotinae, Rhagophthalmidae, and *Pristolycus* & Luciolinae (PP = .75; Fig. 3).

The phylogenetic affiliation of the NA genus *Phausis* remains unclear. Morphological data place *Phausis* with the European genera *Lamprohiza* and *Phosphaenus* (Branham and Wenzel, 2003), but our molecular data leave this question unresolved (Fig. 3). The unique characteristics (i.e. autapomorphies) of *Phausis* only complicate the situation further, and already Green (1959) noted that *Phausis* does not fit well with other species in the Lampyrinae. For example, *Phausis* has a peculiar and uncommon (only documented for *Paraphausis* and *Microphotus*) minute appendage (a vitreous sphere) on the terminal article of its antennae (McDermott, 1964). This is also reflected in its DNA sequence, which shows many unique insertions (e.g. 18S) and base pair positions (e.g. in COI) not found in any of the other lampyrids in this study.

4.2. The effect of taxon sampling and missing data

Although differences in taxon sampling have been implicated as a source of conflict between phylogenetic hypotheses and different levels of phylogenetic accuracy (e.g. Hillis, 1998; Zwickl and Hillis, 2002), our worldwide analysis (65 taxa) produced identical phylogenetic relationships among the NA taxa as did the NA analysis (31 taxa) on its own. This suggests that the taxon sampling in our NA sample was sufficient. A more significant effect on the outcome of a phylogenetic analysis seems to be due to the inclusion of gene segments for which some taxa have missing data. This phenomenon has been well studied for parsimony analyses, where it does not seem to affect phylogenetic

Please cite this article in press as: Stanger-Hall, K.F. et al., Phylogeny of North American fireflies (Coleoptera: ..., Mol. Phylogenet. Evol. (2007), doi:10.1016/j.ympev.2007.05.013

accuracy of the resulting phylogeny (e.g. Wiens, 2003), however, it seems to have a significant effect on the outcome of a ML and/or Bayesian analysis (Lemmon et al., unpublished data). This led us to exclude DNA segments with missing data for more than one taxon from our final alignment. As a result our final data set represents a trade-off between maximizing information (including as many nucleotides as possible) and minimizing analysis bias (reducing the segments with missing data). In the worldwide data set, we included several Asian taxa for which only 16S information was available from Genbank. Ideally, all of our analyses should be based on more than one gene to ensure that our results reflect the phylogenetic relationships of our study species and are not biased by the evolutionary history of a single gene (e.g. 16S), as may be the case for many of the Asian taxa in our study.

4.3. Morphology and molecules

Branham and Wenzel (2003) conducted a phylogenetic analysis of 73 morphological characters for 85 lampyrid species. Our analysis shares 24 of these species. Both datasets support the monophyly of the NA subfamily Photurinae (Fig. 7). However, there are also multiple conflicts between these two datasets including the grouping of *Drilaster, Pterotus, and Stenoclaudius* within Lampyridae in the molecular, but not the morphological analysis. It is possible that, aside from sampling different taxa and employing different phylogenetic algorithms (due to the nature of morphological vs. molecular data), conflicts may result from a high level of convergence in morphological data, observed by Branham and Wenzel (2003; see also Jost and Shaw, 2006).

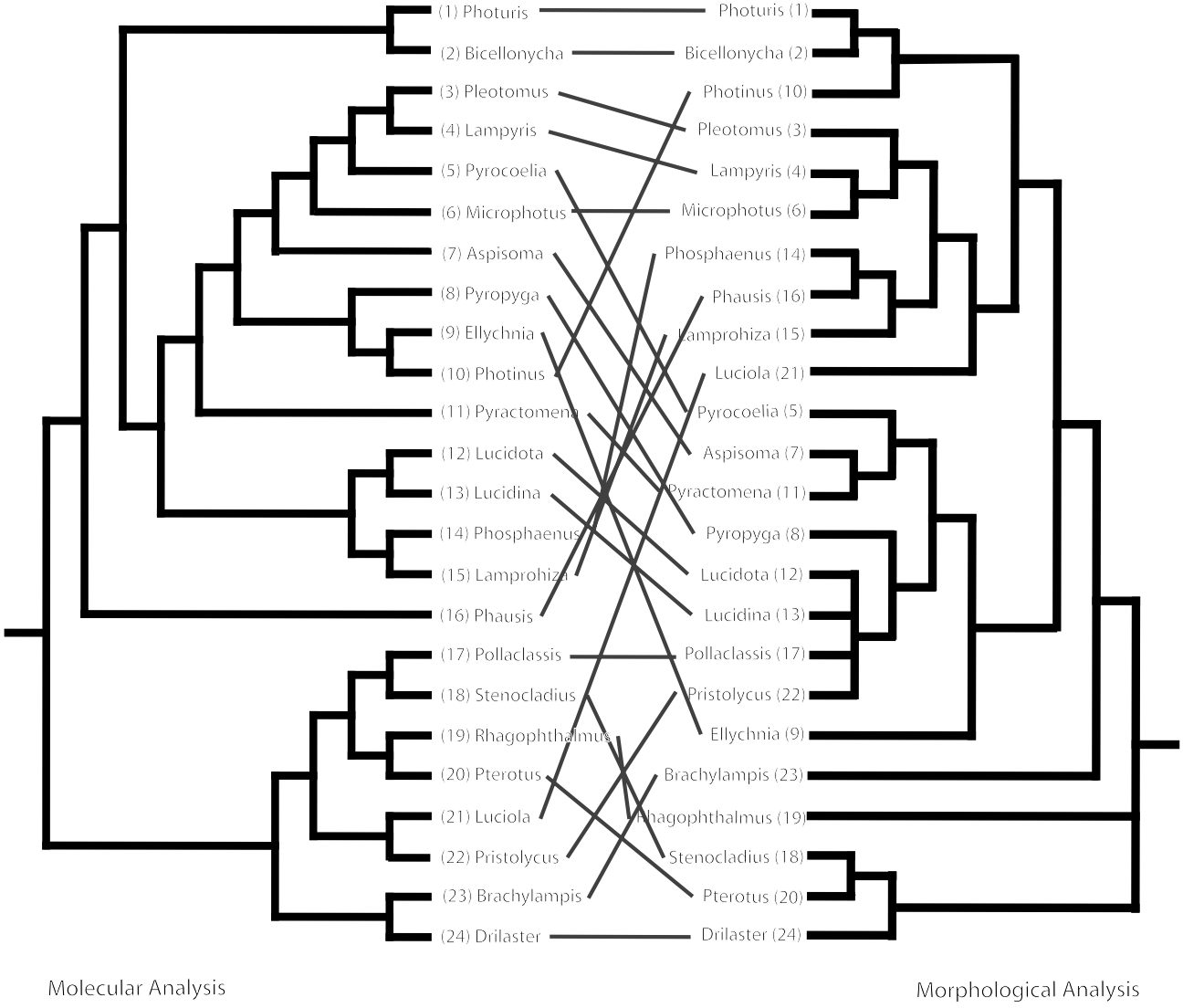

Fig. 7. Morphological (Branham and Wenzel, 2003) and molecular (this study) phylogenies for the 24 lampyrid genera shared by both analyses (all other taxa were pruned from the trees). To facilitate comparison, the 24 taxa were numbered (top to bottom) based on their position on the molecular tree.

Please cite this article in press as: Stanger-Hall, K.F. et al., Phylogeny of North American fireflies (Coleoptera: ..., Mol. Phylogenet. Evol. (2007), doi:10.1016/j.ympev.2007.05.013

4.4. Evolution of sexual signal modes

All known lampyrid larvae produce a faint glow using a paired larval light organ on the eighth abdominal segment, but adult lampyrids vary greatly in the absence, presence, location, shape and use of adult light organs (Branham and Wenzel, 2003). It has been suggested (McDermott, 1964; Sivinski, 1981; Branham and Wenzel, 2003) that luminescence in adult lampyrids is a carryover from the larval stage where it functions as an aposematic warning signal (e.g. De Cock and Matthysen, 2001, 2003), and that such an adult warning signal has been co-opted in many species as a sexual signal. Interestingly, only very few adult lampyrids (e.g. *Robopus*, *Pleotomus*, *Phosphaenus*) use a light organ homologous to the larval light organ (on 8th ventral segment of adult); the adult light organs of most lampyrids are located on the 6th and 7th ventral segment, and may have evolved as a result of sexual selection (see Branham and Wenzel, 2003, for discussion and overview), after one or more initial mutation events causing the expression of light organ genes in the 6th and 7th ventral segments during pupation. Light organ morphology in adult lampyrids shows great variation in size, shape, and exact positioning of light organs on the 6th and 7th ventral segments (Branham and Wenzel, 2003), in neural control (e.g. Carlson, 2004), and in resulting signal patterns (e.g. number and temporal arrangement of flashes: Barber, 1951; Lloyd, 1966), and is likely subject to sexual selection (e.g. Branham and Greenfield, 1996).

The basal position of *Brachylampis* (among others), in their morphological analysis, along with its lack of adult photic organs, led Branham and Wenzel (2003) to conclude that pheromones were the ancestral signal mode in adult lampyrids. Similarly, in our analysis *Brachylampis* is one of several basal taxa that use pheromones (Fig. 4), however, in an equally basal position is *Phausis* (Fig. 4), which produces glows with a light organ on the 7th ventral segment (Branham and Wenzel, 2003). As a result, our analysis suggests several possible scenarios for the evolution of adult light signals. Scenario A requires only a single origin of adult light signals (followed by nine losses), and scenario B requires the least number of changes overall (four independent origins followed by 4 losses; Fig. 5). Scenario B (Fig. 5) is favored under the assumption that losses and gains are equally likely. However, losses of sexual light signals may be much more likely than new gains, as a result scenario A (one gain and nine losses) should be considered. There are at least two more possible scenarios (not shown) that require at least 10 changes, but both of these include even more independent origins compared to scenario B, and so these scenarios would not be favored over scenario B unless gains of light signals are actually considered to be more likely than losses. Note that scenarios A and B (Fig. 5) are the same except for the group that includes the Luciolinae, Rhagophthalmidae, and C*yphonocerus* (see Fig. 4). Under scenario A, these groups share an ancestral sexual light signal, whereas under scenario B, sexual light signals originated independently in these three groups (Fig. 5). These three groups exhibit different display types (flashes, glows, and pheromones/weak glows, respectively: see Fig. 6), which may add additional support for scenario B. Under both scenarios A and B, in contrast, the sexual light signal shared by species of Lampyrinae (except *Pristolycus*) and Photurinae appears to be ancestral, with at least four losses of light (Fig. 5).

The use of light flashes as sexual signals evolved either two or three times in our study group (Fig. 6: scenarios A and B). Both scenarios require a total of six steps (gains and losses), but scenario A should be favored under the assumption that a loss of flashes is more likely than a gain. Both scenarios suggest one origin of flashes in the Luciolinae, and either one (A) or two (B) origins in the Lampyrinae (exc. Pristolycus)/Photurinae group. Interestingly, in *Phausis reticulata* (Fig. 4) both males and females produce glows, and in some instances a female initiates her glow in response to seeing a male glow, rather than starting to glow at the beginning of her activity period (independent of male signal input) as is the case for most other lampyrids that signal with glows (Lloyd, 1997; Branham and Wenzel, 2003). The phylogenetic position of *Phausis reticulata* (see Fig. 4) suggests that the ability to control light emissions may have originated in the common ancestor of Lampyrinae (except *Pristolycus*) and Photurinae, and, through selection, resulted in the fine-tuned temporal control of light emissions required for flashing behavior. Whether flashing behavior originated once (scenario A) in the descendants of this group, or whether it originated twice (scenario B) remains to be investigated. The ability to control the onset of glowing in the common ancestor, seems to favor scenario A. Under strong selection for increased control of light emissions, however, the gain of flashes (from a controlled glow) may be at least as likely as a loss (favoring scenario B).

One of the few groups that show an equally impressive sexual signal diversity (and associated diversity in signal-related organ morphology) are the Ensifera (crickets, katydids and relatives: see Jost and Shaw, 2006, for an overview). Similar to lampyrids, there is a high degree of conflict between morphological and molecular data sets in Ensifera, which has been attributed to a high degree of morphological homoplasy, particularly in those characters related to acoustic organ and ear morphology that are thought to evolve under strong sexual selection (Jost and Shaw, 2006). Similarly, Branham and Wenzel (2003) reported a high level of homoplasy in light organ ($CI = 0.38$) and antennae (chemical signal sensors: $CI = 0.44$) morphology in lampyrids, but they noted that this was considerably less than the level of homoplasy in other morphological characters in their data set (e.g. wing venation: $CI = 0.12$). When plotted onto a pruned molecular phylogeny of the 24 genera shared by both morphological and molecular analyses (Fig. 7), and compared to the pruned morphological hypothesis (Branham and Wenzel, 2003), 29 out of 67 informative morphological characters

Please cite this article in press as: Stanger-Hall, K.F. et al., Phylogeny of North American fireflies (Coleoptera: ..., Mol. Phylogenet. Evol. (2007), doi:10.1016/j.ympev.2007.05.013

had a higher CI when plotted onto the morphological phylogeny (as would be expected since those same characters were used to generate that phylogeny), but 28 characters fit the two conflicting phylogenies equally well (same CI), and 11 characters had a higher CI on the molecular phylogeny. Overall, the evolution of morphological characters required significantly fewer steps on the morphological tree (Wilcoxon matched-pair signed rank test: $p < 0.001$). The average CI for potentially signal-related characters (light organs, eyes, antennae) was higher on both, molecular and morphological phylogenies (0.514 and 0.551, respectively), than the average CI for non-signal related characters (head shape, wing veins and other exoskeleton features: 0.435 and 0.461, respectively). However, these differences were not significant for either phylogeny ($p > 0.1$; for both, location (Kruskal Wallis test) and distribution (Mann Whitney *U*-test), corrected for ties). This suggests that signal related characters as a group are not evolving significantly differently from other morphological characters, but what is needed is a direct test of selection. A detailed quantitative analysis of sensor morphology (eyes and antennae) is presently underway to investigate whether these traits are under selection in the taxa used for our entire molecular analysis.

The molecular phylogeny presented in this study can be utilized to address further questions. For example, why are adult light signals lost during evolutionary history? Are they expensive in terms of metabolic cost (Stanger-Hall and Woods unpublished data), or associated cost of predation (e.g. spiders and bats: Lloyd, 1973; *Photuris* fireflies: Lloyd, 1997; Demary et al., 2006)? What happens to chemical signals when light signals evolve? Are pheromones completely lost (as suggested by Branham and Wenzel, 2003), or are pheromones still produced in the background (but play a lesser role in communication), as suggested by the repeated loss of light signals and reversal to chemical signaling (e.g. flashes to pheromones: *Ellychnia*, *Pyropyga*) in the present study?

4.5. Future challenges

To study the evolution of glows and flashes as mating signals in all lampyrids, representatives of all subfamilies and genera need to be included in the analysis. A large proportion of the lampyrid fauna of tropical America, the presumptive origin and region of greatest lampyrid diversity (McDermott, 1964, 1966), remains unknown. Their study will play a crucial role for our understanding of lampyrid relationships and our efforts to study the evolution of specific traits. The inclusion of Asian, European and American taxa in the present study has given us an idea of several lampyrid relationships and origins, but it will be essential to sample the worldwide lampyrid diversity adequately (across all taxonomic and geographical subgroups) to gain a thorough understanding of their phylogenetic and geographical relationships. Most notably, no DNA sequence data are presently available from African and Australian lampyrids, or, with the exception of *Aspisoma*, from tropical America. Even though our present analysis only allows us a glimpse at the worldwide phylogenetic relationships of the diverse NA fauna and the evolution of their mating signals, we hope to have made an important first step towards achieving this goal.

Acknowledgments

We gratefully acknowledge Joseph Cicero, John Sivinski, Lloyd Davis, Arvin Provonsha, Brian Cassel, Elwin Evans, Riley Nelson, Robert Sites, Rebecca and Rachel Steinberg, Paige Warren, Brian West, and many others for help with the collection of the NA lampyrids used in this study. Thanks also to Raphael De Cock, Ulrich Mueller and M.L. Jeng for sharing their international specimens, and to John Day for sharing his *Lampyris* sequence. Joe Cicero identified the lycid specimen. James Bull provided logistic support, and my (KSH) former REU undergraduate students, Meredith McClure, Laura Reinhard, and Janelle Ortiz, contributed to the laboratory work with great enthusiasm. We also appreciate the insights of Chris Funk regarding the successive ML analysis and Derrick Zwickl, Alan Lemmon, and Tracy Heath regarding the Bayesian analysis. Also thanks to the Hillis/Bull lab group, and to the extended group associated with the lab of Michael Ryan for stimulating discussions and support. Thanks especially to Greg Pauly for comments on an early version of this manuscript. Finally, thanks to all the people who helped collect fireflies over the years or who were involved in any other way. This study was funded by NSF (DEB # 0074953).

References

Baldwin, B.S., Black, M., Sanjur, O., Gustafson, R., Lutz, R.A., Vrijenhoek, R.C., 1996. A diagnostic molecular marker for zebra mussels (*Dreissena polymorpha*) and potentially co-occurring bivalves: mitochondrial COI. Mol. Mar. Biol. Biotechnol. 5, 9–14.

Barber, H.S., 1951. North American fireflies in the genus *Photuris*. Smithson. Misc. Coll. 117 (1), 1–58.

Branham, M.A., Greenfield, M.D., 1996. Flashing males win mate success. Nature 381, 745–746.

Branham, M.A., Wenzel, J.W., 2001. The evolution of bioluminescence in Cantharoids (Coleoptera, Elateroidea). FLA Entomol. 84 (4), 565–586.

Branham, M.A., Wenzel, J.W., 2003. The origin of photic behavior and the evolution of sexual communication in fireflies. Cladistics 19, 1–22.

Carlson, A., 2004. Is the firefly flash regulated by calcium?. Integr. Comp. Biol. 44 220–224.

Choi, Y., Bae, J., Lee, K., Kim, S., Kim, I., Kim, J., Kim, K., Kim, S., Suzuki, H., Lee, S., Sohn, H., Jin, B., 2003. Genomic structure of the luciferase gene and phylogenetic analysis in the *Hotaria*-group fireflies. Comp. Biochem. Physiol. B, Biochem. Mol. Biol. 134 (2), 199–214.

Crowson, R.A., 1972. A review of the classification of Cantharoidea (Coleoptera) with the definition of two new families, Cneoglossidae and Omethidae. Rev. Univ. Madrid 21 (82), 35–77.

Cunningham, C.W., 1997. Can three incongruence tests predict when data should be combined? Mol. Biol. Evol. 14 (7), 733–740.

De Cock, R., Matthysen, E., 2001. Do glow-worm larvae (Coleoptera: Lampyridae) use warning coloration? Ethology 107, 1019–1033.

De Cock, R., Matthysen, E., 2003. Glow-worm larvae bioluminescence (Coleoptera: Lampyridae) operates as an aposematic signal upon toads. Behav. Ecol. 14, 103–108.

Demary, K., Michaelidis, C.I., Lewis, S., 2006. Firefly courtship: behavioral and morphological predictors of male mating success in *Photinus greeni*. Ethology 112 (5), 485–492.

Farris, J.S., Källersjö, M., Kluge, A.G., Bult, C., 1995. Testing significance of incongruence. Cladistics 10, 315–319.

Felsenstein, J., 2004. Inferring Phylogenies. Sinauer Associates Inc., Sunderland Massachusetts.

Green, J.W., 1956. Revision of the Nearctic species of *Photinus* (Lampyridae: Coleoptera). Proc. Calif. Acad. Sci. Ser. 4 (28), 561–613.

Green, J.W., 1957. Revision of the Nearctic species of *Pyractomena* (Coleoptera: Lampyridae). Wasman J. Biol. 15, 237–284.

Green, J.W., 1959. Revision of the species of *Microphotus* with an emendation of the Lampyrini (Lampyridae). Coleopt. Bull. 13, 80–96.

Green, J.W., 1961. Revision of the species of Pyropyga (Lampyridae). Coleopt. Bull. 15, 65–74.

Hillis, D.M., 1998. Taxonomic sampling, phylogenetic accuracy, and investigator bias. Syst. Biol. 47, 3–8.

Huelsenbeck, J.P., Larget, B., Miller, R.E., Ronquist, F., 2002. Potential applications and pitfalls of Bayesian inference of phylogeny. Syst. Biol. 51 (5), 673–688.

Huelsenbeck, J.P., Rannala, B., 2004. Frequentist properties of Bayesian posterior probabilities of phylogenetic trees under simple and complex substitution models. Syst. Biol. 53 (6), 904–913.

Jost, M.C., Shaw, K.L., 2006. Phylogeny of *Ensifera* (Hexapoda: Orthoptera) using three ribosomal loci, with implications for the evolution of acoustic communication. Mol. Phyl. Evol. 38 (2), 510–530.

Kim, J.-G., Kim, I., Bae, J.-S., Jin, B.-R., Kim, K.-Y., Kim, S.-E., Choi, J.-Y., Choi, Y.-C., Lee, K.-Y., Sohn, H.-D., Noh, S.-K., 2001a. Genetic subdivision of the firefly, *Luciola lateralis* (Coleoptera: Lampyridae), in Korea detected by mitochondrial COI gene sequences. Korean J. Genet. 23, 203–219.

Kim, I., Lee, S.C., Bae, J.S., Jim, B.R., Kim, S.E., Kim, J.K., Yoon, H.J., Yang, S.R., Lim, S.H., Sohn, H.D., 2001b. Genetic divergence and phylogenetic relationships among the Korean fireflies, *Hotaria papariensis*, *Luciola lateralis*, and *Pyrocoelia rufa* (Coleoptera: Lampyridae), using mitochondrial DNA sequences. Hanguk Ungyong Konchung Hakhoe Chi 39 (4), 211–226.

Kobayashi, Y., Suzuiki, H., Ohba, N., 2001. Formation of a spherical germ rudiment in the glow-worm *Rhagophthalmus ohbai* Wittmer (Coleoptera: Rhagophthalmidae) and its phylogenetic implications. Proc. Arthrop. Embryol. Soc. Jpn. 36, 1–5.

Lawrence, J.F., Newton, A.F., 1995. Families and subfamilies of Coleoptera. In: Pakaluk J., Slipinski, S.A. (Eds), Biology, phylogeny and classification of Coleoptera: Papers celebrating the 80th birthday of Roy A. Crowson. Muz. Inst. Zool PAN, Warsaw, Poland, pp. 849–861.

Lloyd, J.E., 1966. Studies on the flash communication system in *Photinus* fireflies. Misc. Publ. Mus. Zoology, University of Michigan No. 130, Ann Arbor, Michigan.

Lloyd, J.E., 1969. Flashes of *Photuris* fireflies: their value and use in recognizing species. FLA Entomol. 52, 29–35.

Lloyd, J.E., 1973. Firefly parasites and predators. Colleopt. Bull. 27, 91–106.

Lloyd, J.E., 1997. Firefly mating ecology, selection and evolution. In: Choe, J.C., Crespi, B.J. (Eds.), Evolution of Mating Systems in Insects and Arachnids. Cambridge University Press, London, pp. 184–192.

Lloyd, J.E., 2002. Lampyridae. In: Arnett, R.A., Thomas, M.C., Skelley, P.E., Frank, J.H. (Eds.), American Beetles, Vol. 2. CRC Press, Boca Raton, pp. 187–196.

Maddison, D.R., Maddison, W.P., 2000. McClade v.4. Sinauer Associates Inc., Sunderland, MA.

McDermott, F.A., 1964. The taxonomy of the Lampyridae (Coleoptera). Trans. Amer. Entomol. Soc. 90, 1–72.

McDermott, F.A., 1966. Coleopterorum Catalogus. Supplementa Edita: Pars 9 Lampyridae. W. Junks, W.O. Steele, Gravenhage.

Nylander, J.A.A., Ronquist, F., Huelsenbeck, J.P., Nieves-Aldrey, J.L., 2004. Bayesian Phylogenetic analysis of combined data. Syst. Biol. 53 (1), 47–67.

Ohba, N., 1983. Studies on the communication system of Japanese fireflies. Sci. Rept. Yokosuka City Mus. 30, 1–60.

Ohba, N., 2004. Flash Communication systems of Japanese fireflies. Integr. Comp. Biol. 44 (3), 225–233.

Posada, D., Crandall, K.A., 1998. Modeltest: testing the model of DNA substitution. Bioinformatics 14 (9), 817–818.

Posada, D., Buckley, T.R., 2004. Model selection and model averaging in phylogenetics: advantages of Akaike Information criterion and Bayesian approaches over likelihood ratio tests. Syst. Biol. 53 (5), 793–808.

Ronquist, F., Huelsenbeck, J.P., 2003. MRBAYES 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19, 1572–1574.

Simon, C., Frati, F., Beckenbach, A., Crespi, B., Liu, H., Flook, P., 1994. Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. Ann. Rev. Entomol. 87, 651–701.

Sivinski, J., 1981. The nature and possible functions of luminescence in Coleoptera larvae. Coleopts. Bull. 35 (2), 167–179.

Sullivan, J., Abdo, Z., Joyce, P., Swofford, D.L., 2005. Evaluating the Performance of a successive-approximations approach to parameter optimization in maximum likelihood phylogeny estimation. Mol. Biol. Evol. 22 (6), 1386–1392.

Suzuki, H., 1997. Molecular phylogenetic studies of Japanese fireflies and their mating systems (Coleoptera: Cantharoidea). Tokyo Metro. Univ. Bull. Nat. History 3, 153.

Swofford, D.L., 2002. PAUP. Phylogenetic Analysis Using Parsimony, 4th ed. Sinauer Associates Inc., Sunderland, MA.

Swofford, D.L., Olsen, G.J., Woodell, P.J., Hillis, D.W., 1996. Phylogenetic Inference. In: Hillis, D.M., Moritz, C., Mable, B.K. (Eds.), Molecular Systematics. Sinauer Associates Inc., Sunderland, MA, pp. 407–514.

Viviani, V.R., 2001. Fireflies (Coleoptera:Lampyridae) from Southeastern Brazil: habitats, life history, and bioluminescence. Ann. Entomol. Soc. Amer. 94 (1), 129–145.

Vogler, A.P., DeSalle, R., 1993. Phylogeographic patterns in coastal North American tiger beetles *Cicindela dorsalis* inferred from mitochondrial DNA sequences. Evolution 47, 1192–1202.

Whiting, M.F., Carpenter, J.C., Wheeler, Q.D., Wheeler, W.C., 1997. The *Strepsiptera* problem: phylogeny of the holometabolous insect orders inferred from 18S and 28S ribosomal DNA sequences and morphology. Syst. Biol. 46, 1–68.

Wiens, J.J., 2003. Missing data, incomplete taxa, and phylogenetic accuracy. Syst. Biol. 52 (4), 528–538.

Wittmer, W., Ohba, N., 1994. Neue Rhagophthalmidae (Coleoptera) aus China und benachbarten Laendern. Jpn. Entomol. 62, 341–355.

Zwickl, D.J., Hillis, D.M., 2002. Increased taxon sampling greatly reduces phylogenetic error. Syst. Biol. 51, 588–598.

Please cite this article in press as: Stanger-Hall, K.F. et al., Phylogeny of North American fireflies (Coleoptera: ..., Mol. Phylogenet. Evol. (2007), doi:10.1016/j.ympev.2007.05.013