

A day-flashing *Photinus* firefly (Coleoptera: Lampyridae) from central Panamá: an emergent shift to predator-free space?

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Abstract

Fireflies in the genus *Photinus* are well regarded for their luminescent nocturnal courtship displays. Here we report on a new firefly species, *Photinus interdius*, which is remarkable for its fully diurnal and luminescent courtship protocol. Males slowly flew near the ground searching for receptive females and emitted 800 ms, bright yellow light flashes at 3–4-s intervals. Male flights occurred as early as 13:10 and ceased before 18:00. We sequenced two mitochondrial loci and one genomic locus and combined these with those from 99 specimens representing 45 *Photinus* and 25 related firefly species. Bayesian inference resulted in a well-resolved phylogeny that placed this new species as the closest relative of, but basal to the *Photinus* clade. We propose that the adaptive significance of this extraordinary temporal shift in courtship niche is the outcome of a selective landscape that has optimized the trade-off between reduced predation risk and ease of mate-localization.

Keywords

aedeagus; Bayesian; bioluminescence; courtship; photometry; phylogeny; predation; sexual selection

Introduction

Fireflies of the exclusively New World genus *Photinus* are well-known for their spectacular nocturnal courtship dialogues, wherein flying males broadcast bright flashes of light to locate conspecific, sedentary females, who emit flashed responses with species-specific time delays. As the largest genus in the New World, with over 235 members, *Photinus* is currently represented by 16 species from Panamá, 19 from elsewhere in Central America, and 9 from adjacent Colombia (Blackwelder 1944; McDermott 1966; Zaragoza-Caballero 2015). Here, we describe a new member of *Photinus* from Panamá. The new species, *P. interdius*, is most similar in phenotype, sexual morphology, and in courtship behaviour to the nocturnal *P. pyralis*, the most abundant and widespread

temperate firefly in North America. However, unlike the majority of its luminescent congeners, the courtship of the *P. interdius* **sp. n.** takes place in broad daylight. To our knowledge this is the first report of a day-flashing firefly courtship protocol.

The use of light for communication among individuals generally imposes stringent restrictions upon the evolution of signals. For instance, the receiver of a photic signal must be in the line-of-sight of the sender. This constraint confounds an additional factor that governs light communication: the inverse square law, which states that light intensity decreases as the inverse of the square of the distance from the source. Thus, individuals using light signals, like fireflies, must be in relative propinquity. Further, background “noise” in the form of ambient light precludes the use of photic signals during most of the daylight hours, except in special habitats, such as in caves, burrows, and in deeper marine habitats. This is also the case even with nocturnal fireflies, wherein ambient light from the moon or street lighting may interfere with photic communication. Firefly mating activity is much reduced during a full moon or in the vicinity of sources of street light ‘pollution’ (McDermott & Buck 1959; Ineichen & Rüttimann 2012). Thus, our report on the discovery of a firefly with a fully diurnal luminescent courtship protocol is of special interest.

This study had three objectives. These were to determine if: (1) the newly observed day-flashing firefly was genetically and morphologically distinct; (2) the two allopatric populations of day-flashing fireflies were conspecific; and (3) the phylogenetic position of the new species was basal or derived within the *Photinus* radiation. We then addressed several evolutionary and ecological hypotheses as to why fireflies might opt to avoid the night and to court in the daytime.

Material and Methods

Study sites and collection

During the first week of February 2006, one of us (FVV) discovered a diurnally flashing firefly on Cerro Chucaní, Darien Province, Panamá (8°51'39.81" N; 78°35'35.17" W; alt 780 m). Ninety-five km away, a second putative population of day-flashing fireflies was discovered on 6 March 2010 along the Rio Mendoza at km 13 of Pipeline Road (hereafter, PLR) in Soberania National Park (9°09'54.9" N; 079°45'22.4" W; alt 120 m). These populations were found in primary and late secondary growth rainforest respectively.

Video recordings of flying males were made with a Pentax OPTIO WG-II digital camera. Temperatures ranged between 27.3 and 29°C. Specimens from each population were collected with an aerial net and preserved in 80–100% EtOH.

Photometry and temperature measurements

Measurements of ambient light levels were made in two ways: (1) point measurements at the same location immediately where a courting male had just flashed; and (2) measurements made every 2 m along a 20-m transect, 1 m above the forest floor. At the

Cerro Chucaní site, on 6 February 2006, point measurements of luminous light levels ($n=5$) were made on an overcast day. Point measurements ($n=25$) were made on 10 and 15 March 2015 between 16:30 and 17:30. Transects ($n=9$) were made on 10 March 2015, between 16:00 and 17:30. Values obtained in sun flecks were noted. Both days were mostly sunny, with a few large cumulus clouds typical of the dry season at this time of year.

Light intensities were measured with a LI-190R quantum light sensor attached to a LI-1000 data logger (LI-COR, Lincoln, NB, Canada). Temperatures were recorded at several periods during afternoon courtship activity and at night, between 19:00 and 01:00, using an Omega HH-26 thermometer fitted with a 88L40K thermocouple (Omega, Norwalk, CT, USA).

Wing beat frequency

The dominant audible frequency typically corresponds to the wing beat frequency (e.g., Sotavalta 1952), but higher harmonics may also be present, and sometimes form the dominant or carrier frequency (e.g., Webb et al. 1976). We estimated cps by matching tones from a generator with the dominant carrier sound from video recordings, which was determined by ear (Szynalski 2016).

DNA isolation and sequencing

We isolated DNA from four *P. interdius* **sp. n.** specimens representing two allopatric populations using the DNeasy tissue kit (Qiagen). We amplified three loci: the mitochondrial DNA locus Cytochrome oxidase I (COI), 16S ribosomal RNA (16S), as well as the genomic locus *Wingless* (*Wg*). All primers can be found in Table 1. Polymerase chain reactions (10 μ l total volume) contained 1 μ l of 10 \times buffer, 0.2 mM dNTPs, 0.2 μ l of each 10 μ M primers, 1 U of Perfect *Taq* DNA polymerase (5PRIME) and 1 μ l DNA. PCR amplifications were performed using a thermocycler (Multigene, Labnet) under the following conditions: initial denaturation of 3 min at 94°C, followed by 35 cycles of 30 s at 94°C, 30 s at 51°C and 90 s at 72°C, and a final extension of 10 min at 72°C. The result was treated with 1 μ l of each Exonuclease I and Shrimp alkaline phosphatase (EXO/SAP, Thermo Scientific Fermentas). Reactions were incubated for

Table 1. PCR primers used in this study.

| Locus | Forward primer (5'→3') | Reverse primer (5'→3') | Source |
|-----------|--|--|-------------------------------|
| COI | CI-J-1718: GGAGGATTTGGAAATTGATTA GTTCC | TL2-N-3014: TCCAATGCACTAATCTGCCA TATTA | Simon et al. (1994) |
| 16S | LR-J 13020a: ACGCTGTTATCCCCAAGGTA | LR-N-13398a:CGCCTGTTTAACA AAAACAT | Stanger-Hall et al. (2007) |
| <i>Wg</i> | <i>Wg</i> 26F: TTCGAGTAATCGG CGATRCT | <i>Wg</i> 415R: CGTCCACCACTTGYGTRCGA | This study |

50 min at 37°C followed by a heat deactivation of 10 min at 90°C. The fragments were sequenced with an ABI PRISM 377 automated sequencer using BigDye terminator labelling (Applied Biosystems) by Retrogen. Sequences were cleaned, aligned and analyzed in Geneious R9 (Biomatters).

Phylogenetic analyses

Phylogeny reconstruction was carried out using MrBAYES version 3.2.6 (Huelsenbeck & Ronquist 2001). We ran trees for each individual locus and for all three loci combined. Searches were run for five million generations, sampling every 200 generations and discarding the first 5000 trees (20% burn-in). To generate trees, we used the general time reversible model with invariant sites, gamma rates, and default priors (GTR + I + G). For COI we used 99 specimens from two GenBank popsets (154100342 and 753247081 from Stanger-Hall *et al.* (2007) and Stanger-Hall and Lloyd (2015), respectively, and four *P. interdius* **sp. n.** specimens. For 16S ribosomal RNA we used 37 GenBank specimens from popset 151933850 (Stanger-Hall *et al.* 2007) and two *P. interdius* **sp. n.** specimens. Finally, for Wingless, we used 62 specimens from popset 753246952 (Stanger-Hall & Lloyd 2015) and two *P. interdius* **sp. n.** specimens. To build the tree using all three loci, we partitioned the data by locus and used only 1 individual for each GenBank species, as well as the 4 specimens of *P. interdius* **sp. n.** Because not all 3 loci were available for all species, some individuals had incomplete data. We also built maximum likelihood trees for each gene with RaxML (Stamatakis 2014). Since results were congruent with the Bayesian analyses, we only show results for the latter. The other trees are given in the Appendix to this article as Figs A1–A3. All eight sequences for *P. interdius* **sp. n.** have been deposited in GenBank (KX909930–KX909937).

Morphometrics

Landmarks with well-defined, sharp edges, and with clear and repeatable reference points were selected. Elytron length was measured to the nearest 0.05 mm with calipers from the anterior humeral edge to the apex of the right elytron. Additional measurements of male structures were made using a Wild M8 stereoscope fitted with an ocular grid micrometer. Clear reference points were used after positioning structures with their longitudinal axis perpendicular to the axis of the ocular scale. Pronotal width and length were measured as the widest margin-to-margin and base-to-apex, straight-line distances respectively. Lantern length was measured as the straight-line distance from the anterior edge of the 6th sternite (visible) to the medial notch in the posterior edge of the 7th sternite (visible). Lantern width was taken as the maximal width of the 6th sternite. Lantern area was calculated as the square root of the product of lantern length × width to the nearest 0.2 mm. We measured longest straight-line distances between rigid, sclerotized parts of the base and the apex of the lateral lobes of the aedeagus, which were oriented consistently on the right side in order to align them with the linear ocular scale in the stereoscope. Eye radius, defined here as the distance from the margin of the eye to its apex (highest point) as seen in frontal view,

was measured with calipers. Interocular distance was taken between the inner margins of the eyes when measured at the mid point of the eye in profile at its widest point in the frontal view of the head.

Results

Diurnal luminescent courtship behaviour

Day-flashing males were first observed on Cerro Chucaní at 13:10 on 3 February 2006 under mostly cloudy skies. Flashing displays continued until 17:20. A second instance of day-flashing behaviour was observed 95 km away along the Rio Mendoza on PLR on 11 February 2010. At this site, males were observed to begin courtship at 14:55 under mostly sunny skies, with a few large cumulus clouds typical of the dry season in this part of Panamá. Successive visits to the PLR site revealed that courtships generally took place from mid to late afternoon and ceased well before nightfall (18:30). Searching flights, wherein males sought sedentary receptive females by broadcasting flash signals, were very near the ground, frequently at an altitude of 1–1.2 m. The flight path was relatively slow and linear, at rates varying between 3 and 5 km/h (about walking speed). Flying males emitted a conspicuous buzzing sound, between 91–99 cps, during flight.

The male searching flash pattern consisted of a single, bright yellow flash with a 700–900 ms duration. At 28.9°C, the mean interflash interval (hereafter, IFI) of the patrol flash pattern (i.e., without artificial flash answers) was 4.31 ± 1.2 (mean \pm SD, $n=22$ flashes from 5 individuals). At the moment of flash emission, the male flight path changed abruptly to an upward, 6–8 cm swoop, resembling the letter “J”, much in the same fashion as the common North American congener *P. pyralis* (see the video clip at <https://www.facebook.com/fredric.vencl/videos/g.618366604953262/10203920259829335/?type=2&theater>). After flash cessation, the male hovered briefly for 1–3 s. Searching flight then continued on a 2–6-m path in a relatively straight line, punctuated with “J” gestures and coincident flashes every 3–4 s. Searching often ceased when a male entered a larger (>2 m) light fleck (see below), where upon he landed, and waited several seconds before crossing to the next darker area in the understory to resume the searching part of the courtship protocol.

If the male flash is answered with a single flash produced by an artificial LED with a 1.5–2 s delay, the male courtship protocol changed from the searching to the courtship phase (see the video clip at <https://www.facebook.com/fredric.vencl/videos/g.618366604953262/10203920259829335/?type=2&theater>). Simulated female responses that served to consistently attract males were delivered at an average delay of 1.6 ± 0.3 s (mean \pm SD, $n=53$) as measured from onset of the male flash to the audio report of the artificial response. Males responding to such flashes ceased to perform the “J” flight gesture, and instead, flew toward the location of the answering flash, while shortening the IFI to a mean of 3.63 ± 1.04 s (mean \pm SD, $n=135$ flashes from 10 individuals) at 28.9°C. Males very obviously dimmed their flashes as they entered into the courtship phase of the protocol. One pair (type male and female paratype) was observed to mate

at 16:20 on 6 March 2015. The male and female assumed a 180° position about 8 min after copulation began.

No further flashing from the *P. interdius* **sp. n.** was observed after 17:50, either at the Chucaní or at the PLR sites. Observations were continued at both sites until 22:00–01:00. One single flash species and two glowing *Pyrophorus* (Elateridae) species were captured at the Chucaní and PLR sites, respectively. No *Photuris* fireflies were observed in tree tops and there were no responses to artificial LED flashes, indicating that this predator was not present at either site. Artificial flashes are effective at attracting both *Photuris trivittata* males and females (Vencl et al. 2016). Numerous small bats, most likely *Myotis* sp., were seen at dusk and larger species (perhaps *Molossus* sp.) after dark as they passed near head lamps or through flashlight beams.

Light and temperature levels during courtship

At mid-afternoon flashing behaviour at Cerro Chucaní site under overcast skies occurred with ambient luminous intensities (lx), ranging from 60.23 to 114.1 lx. At the PLR site, ambient luminous intensities where fireflies were seen to emit flashes ranged between 250 and 600 lx and averaged 339.82±88 lx (mean±SD). These light levels correspond roughly to fluorescent light office illumination. Transect illumination values were much more variable. They ranged from 200 to 10 000 lx and averaged 1516.0±2332.8 lx (mean±SD) across the forest floor (Fig. 1). Sun flecks ($n=32$) all had luminosities exceeding 1000 lx. Sunset occurred at 18:25 and 18:29 on 3 February 2006 and 6 March 2015, respectively.

Temperatures at Cerro Chucaní ranged between 24.2 and 27.2°C night and afternoon, respectively. At the PLR site, temperatures were 25.5°C at 22:00 and 29.9°C at 15:00 on 6 March 2015.

Morphological analysis

***Photinus interdius* Vencl et al.**

New species (Fig 2 and 3)

Diagnosis

Pale tan; antennae appearing striped; pronotum with broad, pale margin; shallow, basal groove; broad, medial brownish rose stripe extending from base, extending halfway to apex; distinct, lateral salmon bands; basal-lateral brownish spots; elytra with pale, sub-marginal, diffuse stripes, extending 4/5 to apex; pygidium pointed; length 17–21 mm.

Description

Holotype. Male, Panamá: Colon Province, Pipeline Road, Rio Mendoza, km 13: 9°09'54.9" N, 79°45'22.4" W; date: 3 March 2015 (col: F. V. Vencl) (USNM).

General. Form elongate, parallel-sided, with the elytra 2× as long as broad; habitus dark tan (Fig. 2A); pronotum slightly broader than long; with broad pale margin; a



Fig. 1. Habitat of *Photinus interdius* sp. n. Light regime at 14:12 of the forest understory where males fly and flash in search of females is a mosaic of light flecks of various sizes. Flecks with light intensities levels greater than 1000 lx (circle) would impede male searching flights. Pipeline Road, Rio Mendoza, km 13: 9°09'54.9" N, 79°45'22.4" W; 3 March 2015, Panamá.

broad, black, medial vitta (stripe) extending from base slightly more than half way to apex; medial vitta bordered by salmon-coloured areas; two baso-lateral, dark tan spots; humeral margin complete; sub-marginal, diffuse light-tan vittae; tarsal claws simple; light organs occupying 6th and 7th (visible) sternites; 5th sternite entirely pale white and diaphanous.

Head. Eyes large, separated by shallow, concave depression, the ratio of eye radius to interocular distance was 65%; frons with salmon-coloured maculae (visible in high illumination) near each eye; mandibles robust basally; antennae filiform, weakly compressed: 11 antennomeres: antennomere 2 as long as wide, all others longer than wide; apical end of each antennomere pale white giving antenna striped appearance.

Pronotum. Margins explanate; Slightly broader than long; rounded at apex; disc margin broad, pale-yellow and diaphanous; tan median vitta at base reaching half way to apex, flanked laterally with conspicuous salmon-coloured vittae (No. FA8072; sRGB 250,128,114); a shallow, medial groove extending from base midway to apex; lateral

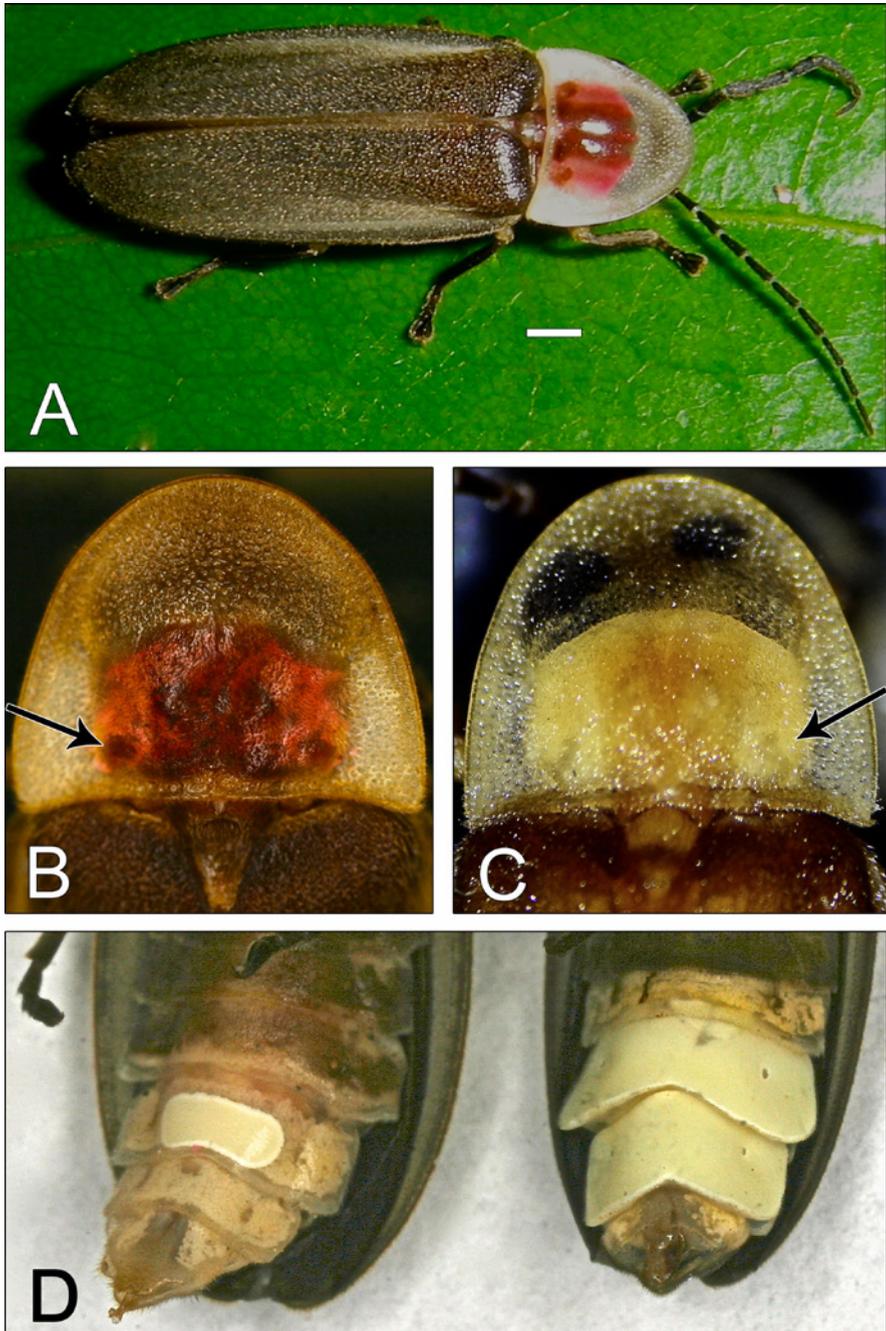


Fig. 2. Habitus of *Photinus interdius* sp. n. (A) Habitus of male (scale=2 mm); (B) dorsal view of pronotum showing baso-lateral spots (arrow) of male from Pipeline Road population; (C) dorsal view of the pronotum from the Cerro Chucaní population showing pale yellowish colour variation and baso-lateral spots (arrow); (D) ventral view of female (left) and male (right) abdominal apex showing lantern dimorphism. Scale=1 mm.

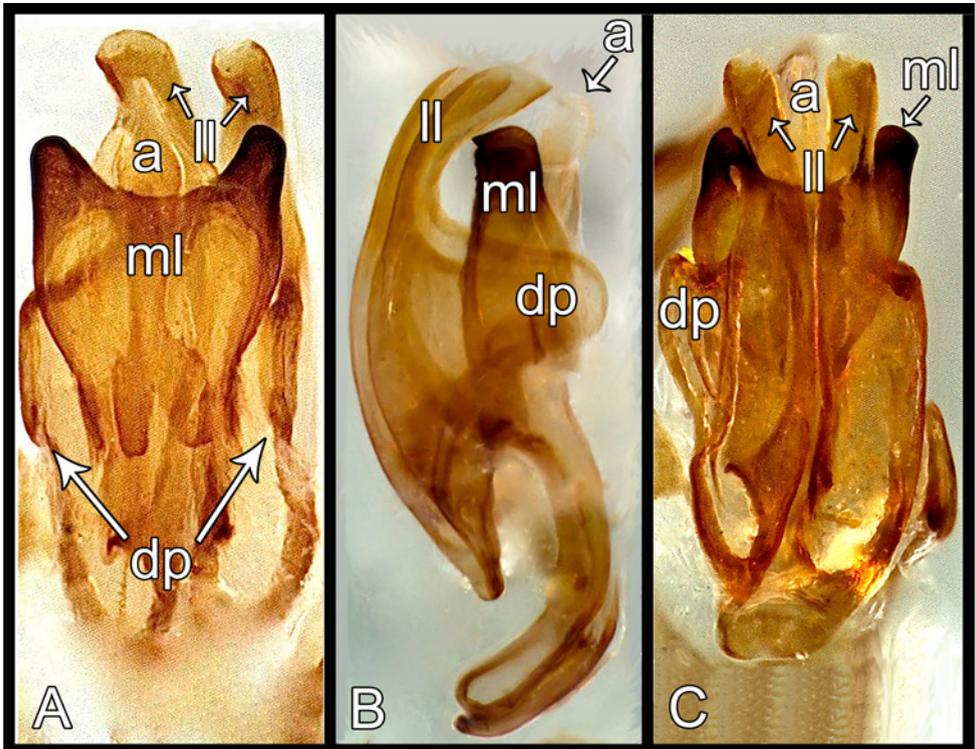


Fig. 3. Aedeagus of *Photinus interdius* sp. n.: (A) dorsal, (B) lateral and (C) ventral views. Abbreviations: a, apex of median lobe; dp, dorsal process of lateral lobe; ll, lateral lobes (arrows); ml, medial lobe. Note: parameres are attached in (B). Length of aedeagus=1.30 mm.

pinkish-brown spots situated laterally at base of each salmon-coloured patch (Fig. 1B); finely punctured throughout.

Scutellum. base dark piceus; apex pale; setose.

Elytra. Tan (dead: No. D2B48C; sRBG 210, 180, 18) and taupe (alive: No. 483C32; sRBG 72, 60, 50); humeral fold complete, acutely margined at edge; a sub-marginal pale, somewhat diffuse vitta extending beyond explanate margin, becoming obsolete toward apex; suture light tan; 4 feeble costae becoming obsolete toward apex; disc finely pubescent with long and short setae; fine, unaligned punctures throughout.

Ventral surface. Sternites 5–7 (visible) convex and explanate, extending beyond the width of the tergites; 5th (visible) sternite entirely pale white and diaphanous; segments 6+7 (visible) entirely luminous, each with lateral stigma (Fig. 1D); pygidium tapering to pointed apex; femora entirely pale yellow; tibia and tarsi brownish; claws simple.

Aedeagus. Median lobe robust, widening apically into two opaque, piceus horns that project dorsally above median lobe, horns sclerotized, with dark pigmented dorsal dentition; apex of median lobe diaphanous and pointed (Fig. 3); lateral lobes prong-like

with truncate ends, exceeding length of median lobe, dorsal processes re-curved to almost embrace above middle of median lobe.

Morphometrics. Length overall: 20.5 mm, with head extended; 18.10 mm (head retracted). Pronotum: length 4.5 mm; width 5.1 mm. Elytra length 13.6 mm. Lantern: length 3.2 mm; width 5 mm; $\sqrt{\text{area}}$ 2.0 mm.

Variation. The Cerro Chucantí population has a mean body length of 18.37 ± 0.33 mm (mean \pm SD, $n=21$). It lacks the salmon-colored pronotal patches of the type, which are instead light-yellowish (Fig. 1C). The scutellum is also light yellowish; elytral suture faintly pale yellow. Ventral surface pale yellow.

Material examined. Pipeline Road ($n=25$); Cerro Chucaní ($n=21$).

Distribution. Lowland rainforest to 800 m, central Panama to Pacific slope.

Etymology. From the Latin *interdius*-in the daytime; by day.

Allotype. Female, Panamá: Colon Province, Pipeline Road, Rio Mendoza, km 13: $9^{\circ}09'54.9''$ N, $79^{\circ}45'22.4''$ W; date: 3 March 2015 (col: F. V. Vencl).

General. In copulo with holotype male. Larger than type.

Head. As in holotype.

Pronotum. As in holotype.

Scutellum. As in holotype.

Elytra. As in holotype.

Ventral surface. Sternites as in type; luminescent area occupying middle of sternite 7 (Fig. 1D); pygidium with apical notch; femora, tibia, tarsi, and claws as in the type.

Morphometrics. Length: 24.1 mm. Pronotum: length 4.9 mm; width 5.5 mm; Elytra length 19.2 mm. Lantern: length 2.2 mm; width 3.5 mm; $\sqrt{\text{area}}$ 2.77 mm.

Vouchers. Holotype: Male, Panamá: Colon Province, Pipeline Road, Rio Mendoza, km 13: $9^{\circ}09'54.9''$ N, $79^{\circ}45'22.4''$ W; date: 3 March 2015 (Vencl) (USNM).

Allotype: Female, Panamá: Colon Province, Pipeline Road, Rio Mendoza, km 13: $9^{\circ}09'54.9''$ N, $79^{\circ}45'22.4''$ W; date: 3 March 2015 (Vencl) (USNM).

Paratypes. Six males, Panamá: Colon Province, Pipeline Road, Rio Mendoza, km 13: $9^{\circ}10.02'$ N; $79^{\circ}45.22'$ W; date: 3 March 2015 (Vencl) (USNM).

Six males, Panamá, Darien Province, Cerro Chucaní: $8^{\circ}51'39.81''$ N; $78^{\circ}35'35.17''$ W, 780 m; date 6 February 2006 (Vencl) (USNM).

Additional material examined. We examined a total of 11 specimens representing three species that shared large size and more than three other traits to *Photinus interdius* **sp. n.**: *P. incongruus* Gorham, syntype male, Panamá, V. de Chiriqui, 4000–6000', BMNH = Natural History Museum London; *P. incongruus* Gorham, syntype male, Panamá, V. de Chiriqui, 4000–6000', B.C.A. col III (2), BMNH; *P. incongruus*

Gorham, syntype male, Panamá, V. de Chiriqui, 4000–6000', B.C.A. col III (2), BMNH = Natural History Museum London; *P. incongruus* Gorham, syntype male, Panamá, V. de Chiriqui, 4000–6000', B.C.A. col III (2); *P. incongruus* Gorham, syntype male, Bugaba, Panamá, Champion, var. ???, BMNH = Natural History Museum London; *P. incongruus* Gorham, syntype male, Bugaba, Panamá, Champion, var. ???, BMNH = Natural History Museum London; *P. congruus* Chevolet, female, MX, Cordova, Salle collec., BMNH; *P. congruus* Chevolet male, BMNH = Natural History Museum London; *P. congruus* Chevolet, female, 132, MX, B.C.A. col III (2), Salle collec., BMNH = Natural History Museum London; *P. congruus* Chevolet, female, Tuxtla, MX, B.C.A. col III (2), Salle collec., var. apnd. Gorham, BMNH = Natural History Museum London; *P. congruus* Chevolet, female, Tuxtla, MX, B.C.A. col III (2), Salle collec., var. apnd. Gorham, BMNH = Natural History Museum London. *P. championi* Chevolet, syntype male, Guatemala, Volcan de Agua, 8500' (Champion), BMNH = Natural History Museum London. *P. championi* Chevolet, syntype female, Guatemala, Volcan de Agua, 8500' (Champion), BMNH = Natural History Museum London.

Remarks

Photinus interdius **sp. n.** is readily distinguished from its congeners by its large size, lack of conspicuous elytral stripes, and by its salmon-coloured pronotum, whose medial stripe begins at the base but does not reach the apex. In addition, the aedeagus, with its horn-like productions emanating from the middle of the median lobe and its prong-like lateral lobes, are quite different from any other photinid described so far. The eye radius /interocular ratio (65%) is closer to that of *P. pyralis* (68%) and much larger than the diurnal, non-luminescent *P. indictus* (38.5%) or *Ellychnia corrusca* (37%).

Phylogenetic analyses

The phylogenetic analyses were consistent between all three loci and the combined dataset (Fig. 4 and Figs A1–A3). Analyses showed that the four *P. interdius* **sp. n.** specimens representing the Cerro Chucaní and Rio Mendoza PLR populations were nearly identical, representing individuals of the same species. The two populations ranged in similarity from 98.75% for the COI locus to 100% for the *Wg* locus. The estimated phylogenetic position of *P. interdius* was basal to the *Photinus* clade with high posterior probability support (Fig. 4 and Figs A1–A3) also showing that the new species is more closely related to members of *Photinus* than any other firefly genus. In other words, *P. interdius* **sp. n.** appears as the sister, basal taxon to the genus *Photinus* in all three trees as well as the combined dataset (Fig. 4, Fig. A1 and the video clip at <https://www.facebook.com/fredric.vencl/videos/g.618366604953262/10203920259829335/?type=2&theater>). Moreover, *P. interdius* **sp. n.** was the only species (out of 45 named species represented by 62 individuals) with one less amino acid at the *Wg* locus, which was caused by a 3-bp deletion, thus supporting its identity within as well as its close affiliation with *Photinus*.

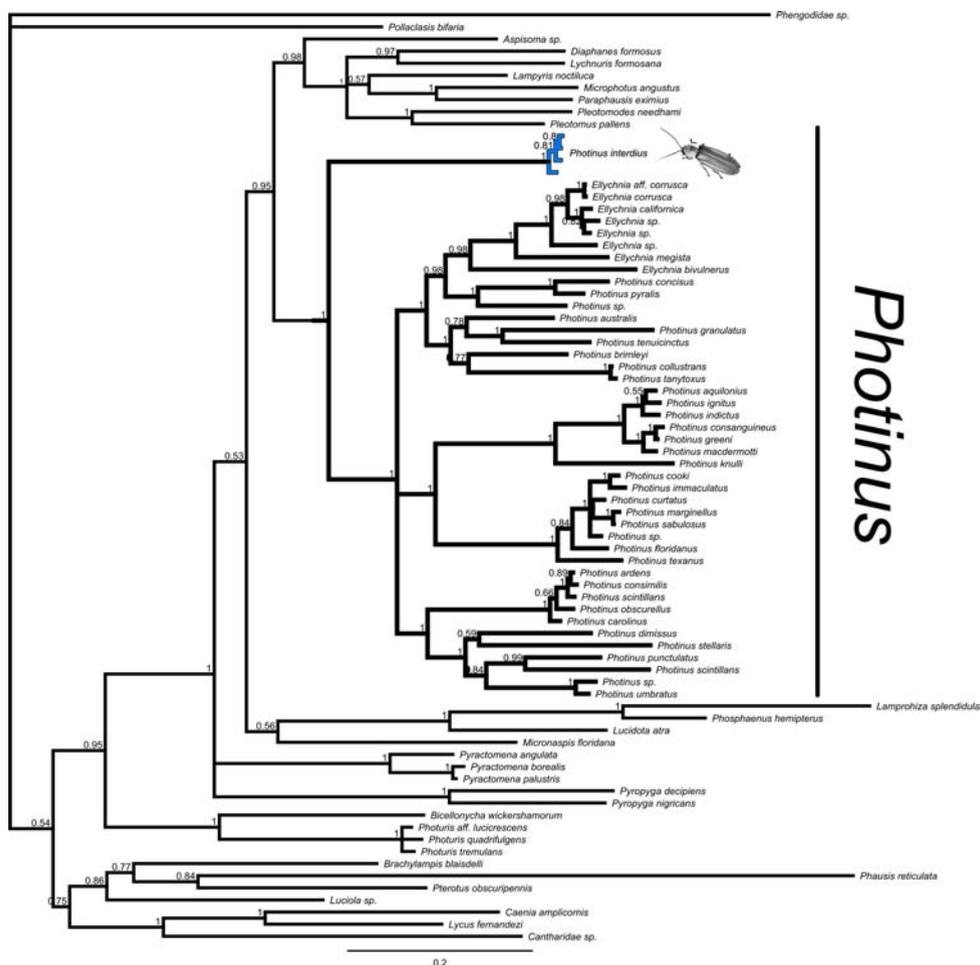


Fig. 4. Phylogenetic relationships of *Photinus* and other New World fireflies derived from the Bayesian analyses of the coding region of COI, 16S RNA and coding region of *Wingless* (*Wg*) and the day-flashing firefly, *Photinus interdius* sp. n. (blue branches; pictured at top). The consensus tree is based on sequence data from 73 GenBank species (limited to 1 individual per species) and four specimens of the new species. Posterior probability supports for each node are shown.

Discussion

The relatively large, closely spaced eyes, with a radius/interocular ratio of over 60% (similar to fully nocturnal species), the non-compressed, filiform antennae, and the fully developed lanterns in both sexes indicate that *Photinus interdius*'s temporal niche shift is of recent origin, mainly encompassing behavioural modifications thus far. The 'dark' fireflies, represented by several *Photinus* species, and by members of *Ellychnia*, lack functional lanterns, more widely spaced, smaller eyes (with convex interocular

spaces), with radius/interocular values less than 50%, and have more elaborate antennae, which are all characteristics of the reversion to pheromone-based courtship.

Evidence from several phylogenetic analyses supports the idea that the firefly ancestor had a diurnal courtship, which employed pheromones rather than photic signals (Branham & Wenzel 2001; Stanger-Hall et al. 2007; Martin 2014). Nocturnal photic courtship appears to have evolved 4 times, and then to have been lost 4 times during the firefly radiation in the North American fauna alone (Brenham & Wenzel 2003). In a more recent analysis of North American fireflies, light as sexual signal evolved one to 4 times and was subsequently lost several times (Stanger-Hall et al. 2007). These gain/loss ambiguities are mostly due to a lack of resolution in some of the basal lineages and to limited taxon sampling. However, all of the diurnal or non-luminescent species are derived. As noted above, not all fireflies have strictly nocturnal courtships. A few common temperate fireflies, like *P. pyralis* and *P. macdermotti* begin their photic courtship searching flights shortly before sunset. These flights often start in darker areas made by the shade of large trees. Light levels at these times range between 3 and 12 lx (e.g., Lloyd 1966), decreasing rapidly to 0.3 lx 45 min after sunset. Moreover, there are well-known instances of “dark” fireflies, such as *P. indictus* and closely related members of *Ellychnia*, that have lost luminescent courtships altogether. So it is with special interest that we report on the discovery of the most basal species yet known in the New World firefly genus *Photinus*, which exhibits a full reversal to a diurnal luminescent courtship protocol, yet lacks the characteristic specializations typical of diurnal species (i.e., lantern loss; reduced, widely spaced eyes; compressed, branched antennae).

The shift to the diurnal courtship niche

The observation of the diurnal courtship of *P. interdius* **sp. n.** raises the question: “Why flash in the day time?” We evaluate two hypotheses to distinguish whether the temporal ‘niche shift’ in courtship exhibited by *P. interdius* **sp. n.** is an adaptation, that is, a behavior that increases fitness. These hypotheses are: (1) temporal ‘character displacement’ to reduce signal interference from other photic species, and (2) reduced mortality by entering a temporal zone that provides predator-free space.

Hypothesis 1 states that if the photic communication channel is fully-booked by numerous flashing species, selection imposed by mate-recognition would favour a shift to a less noisy or vacant bioluminescent niche. The number of sympatric species with the potential to cause signal interference increases with species range (Lloyd 1966). To reduce signal interference, most of these sympatric congeners are thought to show divergence in signal characteristics, such as flash length or inter-flash period, especially among those that have single flash courtship patterns. Moreover, ‘dark’ *Photinus* (i.e., diurnal species courting without interfering luminescence) have higher than average numbers of sympatric congeners, often more than 10 (Stanger-Hall & Lloyd 2015). However, because the night time at both *P. interdius* **sp. n.** sites is nearly devoid of fireflies, or any other luminescent beetle species, the photic ‘noise’ hypothesis is not a satisfactory explanation for the shift in courting niche of *P. interdius* **sp. n.**, at least considering its current distribution.

Hypothesis 2 posits that *P. interdius*'s shift to day-flashing afforded a refuge from historically persistent and intense nocturnal predators. Considerable evidence indicates a crucial role for nocturnal predators in firefly evolution (Branham & Wenzel 2001, 2003; Sagegami-Oba et al. 2007). This evidence also lends support to the potential existence of diurnal predator-free space. For example, bats are suspected to be a major predation pressure on nocturnal fireflies (Lloyd 1989; Moosman et al. 2010; Vencl et al. 2016). Diurnal fireflies, such as *Lucidota atra*, *L. punctata*, and *Ellychnia* spp. as well as crepuscular fireflies, like *Photinus macdermotti* and *P. marginellus*, avoid bats altogether. Diurnal and nocturnal spiders are also known to be firefly predators (Lloyd 1973). Crepuscular fireflies are less susceptible to predation by orb-weaving spiders because they construct their webs after dark and then dismantled them before morning (Lewis et al. 2012). Finally, there are firefly predators that specialize on other fireflies (Lloyd 1965). By responding to courtship signals, the predatory Panamanian firefly, *Photuris trivittata*, whose females attract and consume heterospecific male fireflies, are strictly nocturnal (Vencl et al. 2016). Temperate *Photinus* and *Lucidota* fireflies are known to synthesize lucibufagens (LBGs), which are steroidal pyrone toxins that disrupt the Na^+/K^+ pump, an ion transporter whose function is absolutely essential for animal survival. LBGs have been shown to be repugnant and emetic toxins that thwart birds and spiders (Eisner et al. 1978). *Photuris* females are unable to synthesize their own LBGs and must sequester them from their *Photinus* and *Lucidota* prey (Eisner et al. 1997). LBGs may account for *L. atra*'s unacceptability to birds and spiders (Gronquist et al. 2006). It has been proposed that because the ancestor of *L. atra* was at high risk vis à vis *Photuris* predation, it switched to diurnality, lost its luminescence, and thus escaped *Photuris* predation, without compromising its defended status with respect to other predators through possession of lucibufagens (Gronquist et al. 2006). Although none were directly observed in *P. interdius* sp. n. habitat, *Photuris trivittata* does occur at the same time of year in nearby secondary forest (Vencl et al. 2016).

In further support of the predator-free space idea, heavy chemical defences could account for *P. interdius*'s leisurely flight and conspicuous photic broadcasting during the day. It is known that unpalatable butterflies fly more slowly and regularly (in undulating straight lines) compared to their palatable, undefended counterparts, who are known for their more erratic, hard-to-catch rapid flight paths (Chai & Srygley 1990). The slow, regular flight path, a slow wing beat frequency (around 95 cps), large size, and aposematic (warning) coloration of *P. interdius* sp. n. are traits often possessed by chemically well-defended, unpalatable diurnal insects (Poulton 1890; Cott 1940). There may be conflicting selection on flight speed, flash emission rate, and mate detection during searching phase. Optimally, male flight speed should be slow and flash emissions high to locate receptive females, providing predation pressure is weak. To minimize successful predator attacks, we predict that if predation pressure is high, males should cover greater distances between fewer flash emissions.

In sum, our observations suggest that by switching to the diurnal courtship niche, *P. interdius* sp. n. encounters fewer specialized predators, such as bats and *Photuris femme fatales*. We suggest that the reason for courting during day light and using light intensity to trigger activity is to optimize the trade-off between predation risk and

efficiency of mate localization in the understory light fleck micro habitat. However, the benefit of lower mortality due to predation may be off-set by lower efficiency in mate localization due to ambient light interference.

The Neotropics as nursery or museum for Photinus fireflies?

Many diverse lineages originated in tropical climates and related temperate taxa are often nested within tropical clades, which suggests that they diverged from older tropical groups (e.g., Jablonski et al. 2006). It is generally thought that fireflies originated in the tropics and then spread from there to colonize more temperate habitats as these appeared in the Cenozoic (Grimaldi & Engel 2005). Our phylogenetic analyses indicate that *P. interdius* **sp. n.** is genetically most affiliated with, and basal to the *Photinus* clade, which has its greatest diversity in the Neotropics (Green 1956; McDermott 1964, 1966).

Further evidence supporting this hypothesis is provided by many morphological traits, the most important of which are those of the aedeagus. In his revision of *Photinus*, Green (1956) divided the genus into two divisions based on aedeagal morphology. Species lacking sclerotized baso-ventral projections belonged to the Division I, while those with baso-ventral projections of the median lobe were placed in Division II. Several species in Division I also have small branches or projections stemming from the lateral lobes, which tend to converge over the median lobe (e.g., *P. texanus*, *P. cooki*). More elaborate, convergent projections of the lateral lobes are seen in *P. interdius* **sp. n.** Based on our analysis, it appears that baso-ventral projections were lost and lateral lobe projections were retained in the members of Green's Division I, which we recovered as the derived, monophyletic *texanus* clade. Median lobe baso-ventral projections are also retained in *Macrolampris* and *Ellychnia*. Further, according to our analysis, *Ellychnia* is nested within *Photinus*, *sensu lato* (see Stanger-Hall & Lloyd 2015). Because *P. interdius* **sp. n.** has both median lobe baso-ventral and lateral lobe dorsal projections, we consider these traits to be symplesiomorphies for *Photinus*, which do not necessarily pre-date adult luminescence.

To reconcile the various factors outlined above that may have contributed to the selective landscape fostering *P. interdius*'s shift to the diurnal courtship niche will require: (1) more field work to determine geographic range; (2) chemical analyses to determine whether there are LBGs or other compounds that serve as defences; and (3) surveys of *P. interdius* **sp. n.** predators. Here we have provided a robust phylogenetic hypothesis for the genus *Photinus* with the basic aim of clarifying its relationship to this new species. In sum, our findings support the hypothesis that the diurnal flash communication system of *P. interdius* **sp. n.** represents a compromise between optimizing mating success and minimizing predation risk.

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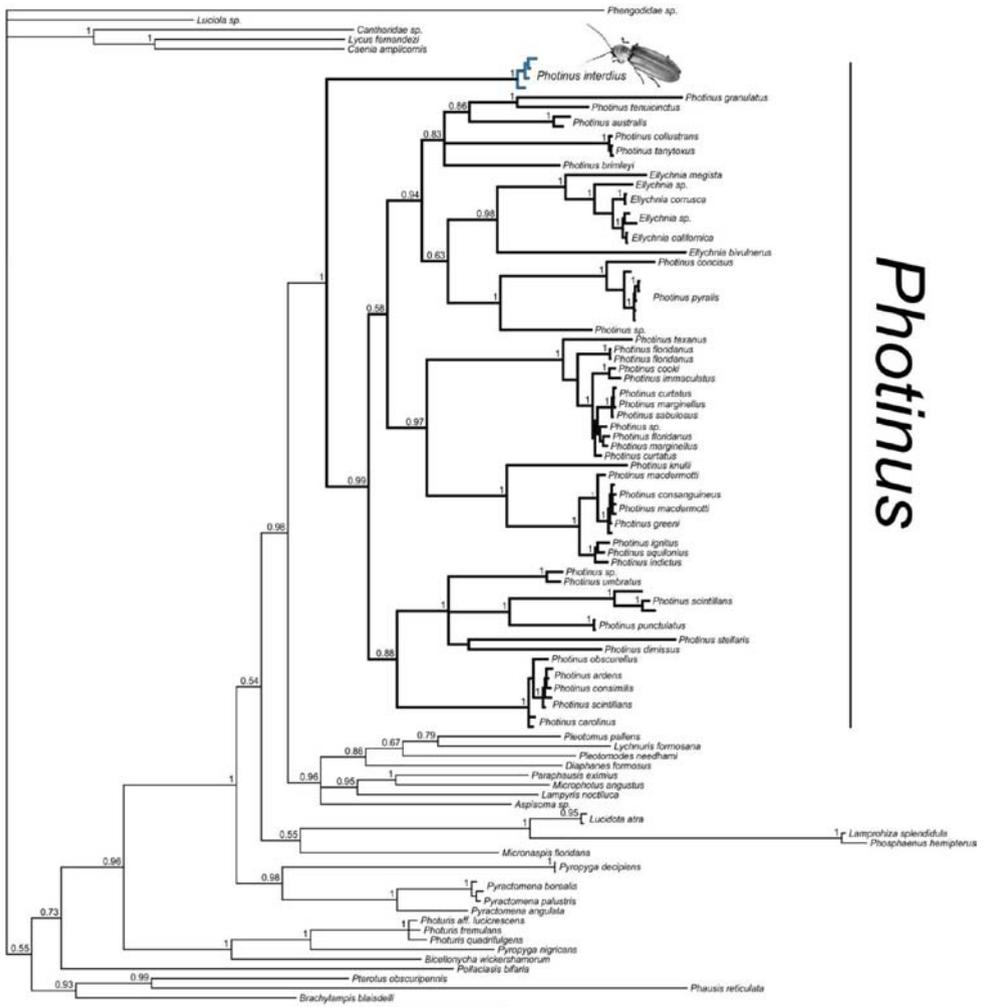


Fig. A1. Phylogenetic relationships inferred from the Bayesian analyses of the coding region of COI between the day-flashing firefly, *Photinus interdius* sp. n. (blue branches; pictured at top), the members of the genus *Photinus*, and other New World fireflies. The tree is based on sequence data from 99 GenBank specimens and four specimens of the *Photinus interdius* sp. n. Statistical measures of posterior probability support for each node are shown. Species names with replicate specimens were removed for clarity.

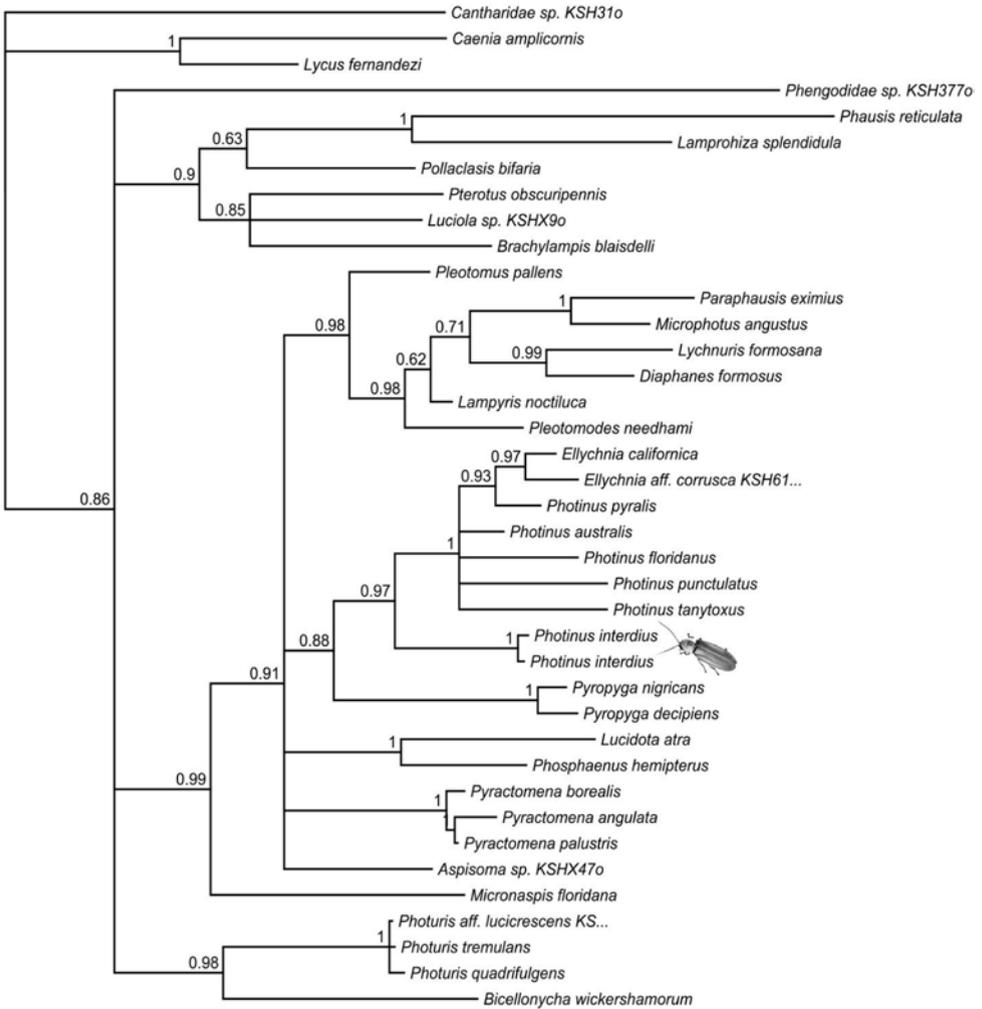


Fig. A2. Posterior probability tree using coding region of 16S ribosomal RNA gene from 37 GenBank specimens and two specimens of the *Photinus interdius* **sp. n.** The MrBayes search was conducted as in Fig. A1.



Fig. A3. Posterior probability tree using coding region of Wingless (*Wg*) from 62 GenBank specimens and two specimens of *Photinus interdius* sp. n. The MrBayes search was conducted as in Fig. A1.