Ohio Biological Survey Notes 9: 16–34, 2019. © Ohio Biological Survey, Inc.

Life History and Updated Range Extension of *Photinus scintillans* (Coleoptera: Lampyridae) with New Ohio Records and Regional Observations for Several Firefly Species

Lynn F. Faust[1], Laura S. Hughes[2], Mark H. Zloba[3], and Heather L. Farrington[4]

[1]Lynn F. Faust, 11828 Couch Mill Rd, Knoxville, TN 37932, TNLFaust@gmail.com; [2]Laura S. Hughes, 365 Shawnee Loop South, Pataskala, Ohio. LS205302@ohio.edu; [3]Mark H. Zloba, Ecological Manager, Cincinnati Museum Center, Edge of Appalachia Preserve System, 4274 Waggoner Riffle Rd., West Union, Ohio 45693, MZloba@cincymuseum.org; [4]Heather L. Farrington, Cincinnati Museum Center, Curator of Zoology, 1301 Western Avenue, Cincinnati, Ohio 45203, HFarrington@cincymuseum.org.

Abstract: *Photinus scintillans* (Say) has long been considered the *Photinus* species with one of the smallest ranges in North America. In field studies conducted between 2016 and 2019 in Ohio and Indiana, we discovered new, thriving *P. scintillans* populations, tripling the east-west range from 550 km to 1820 km when combined with more recent collection records by firefly researchers Lloyd, Stanger-Hall, and Lower. We describe in new detail flight behaviors, nocturnal timing of activity, flash pattern, lantern coloration changes, courtship, and mating habits. We present the first evidence of the presence of spermatophore-producing spiral glands and prolonged mating with the brachypterous females; oviposition behaviors; larval eclosion and appearance; and seasonality with habitat variations and commonalities. We provide the first report with photos of possible phoresy by a springtail (Collembola) on a firefly. In addition, we offer new Ohio state (and nearby Indiana and Kentucky) firefly records, including the extremely rare *P. acuminatus* Green, and provide observations of unusual behaviors in additional Lampyridae species. This study, which involves multiple citizen scientists in several states (please see acknowledgements), aims to encourage discovery of additional new populations and increase the understanding of firefly life history, conservation, and the conditions most likely to favor the survival and appreciation of these charismatic insects.

Keywords: phoresy, springtail, brachypterous, Low Pink Winkers, *Photinus acuminatus, Phausis reticulata*

Introduction

Photinus scintillans (Say) (Coleoptera: Lampyridae) is an inconspicuous and therefore often overlooked or misidentified *Photinus* (Green 1956) species with a single-flash male courtship flash pattern, emitted just above the forest ground cover at dusk, much like many other North American firefly species (Lloyd 1966, Stanger-Hall and Lloyd 2015, Faust 2017). In early firefly studies (Williams 1917, Hess 1920) and in Ohio-specific firefly surveys (Hazard 1929, Marvin 1965), the specimens and associated data of *P. scintillans* and *P. marginellus* LeConte, which frequently fly together, were often mixed together and confused. *Photinus marginellus* (and *P. curtatus* Green [see Green 1956, Lloyd 1967, Lloyd 2001, Faust 2017]) and *P. scintillans* are of similar size and appearance and are often sympatric with each other; their flash patterns and timing of displays (at dusk) are similar, and they are thus easily confused. It is thought that there is one generation per year; eggs are laid in June and July and develop through the autumn to the fourth or fifth instar in northern regions, at which stage they remain dormant until spring.

Even before these twentieth-century studies, *P. scintillans* had an uncertain past. Say (1825) determined that *P. scintillans* (then *Lampyris scintillans*), with a "length nearly 3/10 of an inch," was a different species from the much larger, often sympatric *P. pyralis* (L.). Unfortunately, Say's male holotype, along with many other specimens from his collection, was lost to dermestid beetles and other pests during his illness at the end of his life and after his death at age 47 (Mawdsley 1993). At the time of all of these early studies, there was a lack of understanding on the importance of aedeagal dissections, which readily separate *P. marginellus* and *P. scintillans*. In his encompassing work, Green (1956) illustrated and stressed the importance of confirming differences in the structure of the aedeagi between similar *Photinus* species. He separated the North American *Photinus* species into Division I and II primarily based on aedeagus structure: Division I *Photinus* species have uniquely different aedeagi, whereas Division II *Photinus* share more similar aedeagal structures. Green (1956) demonstrated that even though *P. marginellus* and *P. scintillans* males are superficially alike, *P. marginellus* is classified by its aedeagal structure in Green Division I, whereas *P.*

scintillans belongs to Division II. Green (1956) also reconfirmed that *P. scintillans* females are brachypterous (flightless with shortened, ineffective wings), whereas *P. marginellus* females are fully alate (winged and capable of flight). McDermott (1911, 1914) made progress in understanding the species-specific and sex-specific nature of flash behaviors and illuminated the slight external flash differences between these two commonly confused species. In Lloyd's (1966) and Stanger-Hall and Lloyd's (2015) works on *Photinus*, it was documented and quantified that *P. marginellus* flashes every 4–5 sec, while *P. scintillans* flashes every 2–3 sec at half the flash duration of *P. marginellus* (at similar temperatures).

Further complicating the ambiguous field identification of *P. scintillans* are other sympatric dusk-active, often low-flying *Photinus* species with single-flash flash patterns, including *P. australis* Green*, P. curtatus* (refer to Fig. 7)*, P. pyralis*, and *P. sabulous* Green. Aedeagal and subtle flash pattern differences separate these similar species.

We used male aedeagal structure and female brachyptery to unambiguously identify *P. scintillans* in this study. For identification in the field, we used the timing, flash color, and appearance of the congregated male display, the quick species-specific flash, the manner of low flight, and the characteristic habitat.

There have been few field studies of *P. scintillans* in which species certainty was assured. Lloyd (1966) and Faust (2017) presented brief sketches describing limited natural history on *P. scintillans*. However, this species has been used in many visual and light-emission studies (Seliger and McElroy 1964; Seliger et al. 1982; Lall 1993, 1994; Hall et al. 2016) tracking reaction to light levels and evolution of signal color. Recent opsin studies, also using *P. scintillans* (Sander and Hall 2015, Martin et al. 2015) have looked into the evolution of these specialized, light-sensitive photoreceptor proteins that perceive color and their phylogenetic implications (Stanger-Hall et al. 2007, Stanger-Hall and Lloyd 2015, Sander et al. 2017). Branchini et al. (2017) are studying the biochemical implications of the orange-pink flash coloration discussed and illustrated in this paper. Despite these studies, there is still surprisingly little known about the natural history and behavior of *P. scintillans*.

Studies by Wing (1984, 1985) and later Lewis and Wang (1991) offered that *Photinus* fireflies go through two stages of copulation: stage 1 (male superior, initial contact) and stage 2 (tail to tail, when the spermatophore, if present, is transferred). Wing (1985) compared a *Photinus* species with winged females to a different *Photinus* species (not *P. scintillans*) with the more uncommon brachypterous females. We present new information on the mating stages and copulation durations exhibited by *P. scintillans* that differ from Wing's results and explore the possible reasons for this discrepancy.

Lloyd (1966) states that of all the Nearctic *Photinus* species, *P. scintillans* has the smallest range. His range map, still considered the primary reference for these populations, shows a discrete range encompassing areas in and around eastern Pennsylvania, with an additional population near the Monongahela River in western Pennsylvania. Years later, Lloyd found small, disjunct populations along the Meramec and Roaring Rivers in Missouri and in Lafayette, Indiana, at Purdue Horticultural Park, which he suspected were possibly escapees from travel trailers and nursery stock sent from the east, respectively (J. E. Lloyd, personal communication). Stanger-Hall and Lloyd (2015) later revisited these same geographic outlier sites to collect specimens, yet provided no site or explicit collection details in their comprehensive flash evolution study. Both Lloyd and Stanger-Hall provided those unpublished details for this study. Faust (2017) hinted that robust, darker-colored populations that tentatively appeared to be *P. scintillans* had recently been discovered in far eastern Indiana in 2016.

Since there has historically been confusion of the range and life histories of *P. scintillans*, the two main goals of this study were to determine the range and habitats of *P. scintillans* and to further elucidate the natural history of adult display and courtship behaviors, as well as documenting mating durations, oviposition preferences, and the timing from egg to larval eclosion. To facilitate discrimination and understanding of *P. scintillans* from sympatric species, we discuss the behaviors in the wild of *P. scintillans* along with observations from other firefly species.

During this study, we also observed and confirmed several new Ohio state and regional (Indiana and Kentucky) records and documented several unusual firefly behaviors.

Materials and Methods

Study sites and years. Our primary studies took place at three sites (two in Ohio and one in Indiana) over a period of three years: Whitewater Township, Franklin Co., Indiana, 39.333600°N, 84.889447°W at 295 m, June 2016-2018; Monroe Co., Sardis, Ohio, 39.61590°N, 80.93720°W at 214 m to 305 m, July 2017, 2018; and Washington Co., Ohio, 39.498369°N, 81.247162°W at 212 m, June and July 2018. In 2019, an additional fourth site was found at Cincinnati City Parks' California Woods Nature Preserve in Hamilton County, Ohio, 39.07704°N, 84.42165°W at 183 m. We also added several sites to the recorded *P. scintillans*

range (Green 1956, Lloyd 1966) from Stanger-Hall and Lloyd 2015 (and Lloyd and Stanger-Hall, personal communication): Roaring River State Park, Barry Co., Missouri, 36.7267°N, 91.8736°W, 337 m on June 1, 2003; Meramec State Park, St. Louis Co., Missouri, 38.2067°N, 91.1025°W, 174 m on June 9, 2003; Purdue Horticultural Park, Tippecanoe Co., Lafayette, Indiana, 40.4122°N, 86.9369°W at 183 m July 15, 2002; and Bucks Co., Doylestown, Pennsylvania, 40.3350°N, 75.3130°W at 115 m, May 31, 2010. Faust surveyed a new site in Sussex Co., New Jersey, 41.1191°N, 74.8221°W. We include additional sites and reconfirmation of existing sites in the historic range region (Green 1956, Lloyd 1966) that still have robust *P. scintillans* populations in Berks Co., Pennsylvania, 40.5379°N, 75.7865°W at ≈173 m; Union Co., Pennsylvania, 40.9386°N, 76.8352°W at ≈209 m; Lebanon Co., Pennsylvania, 40.439592°N, -76.598503°W 170 m; and Montgomery Co., Maryland, 38.9839°N, 77.0474°W at ≈68 m. These locations were recently visited by Sarah Lower (personal communication) and are also included on this updated (from Lloyd 1966) range map.

Table 1. Vegetation table for the three *P. scintillans* study sites in Indiana and Ohio.

Family	Specific epithet	Common Name	Indiana Franklin Co.	Ohio Monroe Co.	Ohio Washington Co.
Aceraceae	*Acer saccharum* Marshall	sugar maple	•	•	•
	Acer rubrum L.	red maple		•	•
Annonaceae	*Asimina triloba* (L.) Dunal	pawpaw		•	
Apiaceae	*Hedera helix* L.	English ivy	•		
Asteraceae	*Ageratina altissima* (L.) R.M. King & H Rob	tall eupatorium	•		
	Symphyotrichum sp. L.	asters	•		
Caesalpiniaceae	*Cercis canadensis* L.	redbud	•		
Caprifoliaceae	*Lonicera maackii* (Rupr.) Maxim.	bush honeysuckle	•		
Cupressaceae	*Juniperus virginiana* L.	Eastern red cedar	•		
Dryoperidaceae	*Polystichum acrostichoides* (Michx.) Schott	Christmas fern		•	
Fabaceae	*Robinia pseudoacacia* L.	black locust	•		
Lauraceae	*Lindera benzoin* L.	spicebush		•	•
Malvaceae	*Tilia americana* L.	American basswood		•	
Moraceae	*Morus rubra* L.	red mulberry	•		
Oleaceae	*Fraxinus americana* L.	white ash	•		
	Fraxinus pennsylvanica Marshall	green ash	•		
	Fraxinus quadrangulata Michx.	blue ash	•		
Ulmaceae	*Celtis occidentalis* L.	hackberry	•		

Equipment and field techniques. A commonly used marker in firefly research is the time of flash display initiation or cessation: before, at, or after local sunset. The minutes before or after local sunset that flashing first and last occurs is generally species-specific (Lloyd 1966), though also influenced by population density and cloud or forest cover conditions affecting ambient light. *Photinus scintillans* has been reported to initiate flashing about 60 min prior to sunset (Lloyd 1966), and we arrived at our field sites 90 minutes prior to sunset to record flashing behavior. Specimens were hand- or net-caught with our headlamp aimed directly at the flashing target specimen to assure capture of the correct specimen. For Monroe and Washington counties, Ohio, we used Matamoras, Ohio, sunset times: https://sunrise-sunset.org/us/matamoras-oh/2018/6. For Franklin Co., Indiana, we used West Harrison, Indiana, sunset times: https://sunrise-sunset.org/search?location=west%20harrison,%20IN&year=2018&month=6#calendar.

Descriptions and measurements were made with Tresna digital calipers while examining the specimens through an Omano OMVT stereo microscope and a Nikon SMZ645. Voice recordings were made with an LG4 Android, iPhone4S, and iPhone5. Photographs were taken with Sony Cybershot 1080, Olympus TG4, Canon 5DSR with 100 mm macro lens, Samsung SM-

G930V cell phone camera, Motorola Droid RAZR HD camera, and a Sony HDR-SR11 camera. For additional comparisons, dissections, and identification when we worked as a group at Eulett Center at Cincinnati Museum Center's Edge of Appalachia Preserve System, we used a Nikon SMZ645 dissecting microscope with C-W 10xB/22 lens or a Nikon SMZ800 dissecting microscope attached to a Sony HD Handycam camera viewed through a Sony Bravia television screen.

Captive conditions. Two ovipositing females and the eggs they produced were kept in plastic rearing containers (10 cm long x 4.5 cm high with five 0.5 cm slits cut into the lid) with a small amount of native soil, leaf litter, local moss, and a thin apple slice, misted occasionally. One of these females was kept under natural photoperiod, humidity, and temperature with the lid removed for daily inspection. The other female was kept under laboratory conditions of constant temperature of 21°C and fluorescent lighting nine hours a day with the lid seldom removed.

Degree days. First developed in the 1960s to aid U.S. farmers in timing their corn plantings, and later expanded to prediction of insect activity, the 86/50°F (30/10°C) modified corn-growing degree-day formula (traditionally given in Fahrenheit) with a March 1 start date (modified Growing Degree Days, mGDD/modified Growing Degree Days Celsius, mGDDC) was used to provide degree-day parameters for the three sites (as detailed in Faust and Weston 2009, Faust 2017, and Faust and Forrest 2017). Celsius conversion of this same formula, 30/10°C (mGDDC) (using Fahrenheit value * 0.56), will follow each value in parentheses. Weather stations used for the sites were: Brookville, IN #121030 for the Franklin Co., Indiana, site and Hannibal Locks #333500 for both Washington and Monroe counties, Ohio. Weather stations used for historical data on the newly included outlier and historic sites with dates provided were West Plain, MO #KUNO, Lafayette Purdue AP, IN #129424 and Sellersville, PA #367938 found at http://climod.nrcc.cornell.edu/.

DNA analysis. Two fireflies collected from Indiana and one from Monroe Co., Ohio, were analyzed using DNA barcoding methods to verify species identity. DNA was extracted from three legs on one side of the body via phenol/chloroform extraction, and the remainder of each specimen was retained as a voucher. A portion of the COI locus was amplified using the LCO and HCO primers as described in Stanger-Hall and Lloyd (2015). DNA fragments were sequenced on an ABI 3130 Genetic Analyzer at Cincinnati Museum Center. Resulting sequences were run through a GenBank nucleotide BLAST search on the NCBI website (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

Voucher and Behavior Specimens. DNA and behavior vouchers and new state records can be found at Geier Center of Cincinnati Museum Center (DNA); the Edge of Appalachia Preserve System, Eulett Center; the University of Florida (James E. Lloyd collection); and the private collections of Lynn Faust and Laura Hughes. The recently collected Ohio *Photinus acuminatus* (see results) is kept in the Stanger-Hall Lab collection at the University of Georgia.

Results

Updated range extension. By combining our four sites with new additional data generously provided by Lloyd, Stanger-Hall, and Lower (personal communications), we show that the documented range of *P. scintillans* now stretches at least 1820 km from southeastern New York (73.8°W) to Roaring River, Missouri (93.8°W; Fig. 1). While the range has tripled in east–west extension, it remains largely unchanged in its north–south distribution (as shown in Lloyd 1966) from 36.7° N to 41° N, stretching nearly 340 km and encompassing a much greater range than the 39° north latitude of our three study sites.

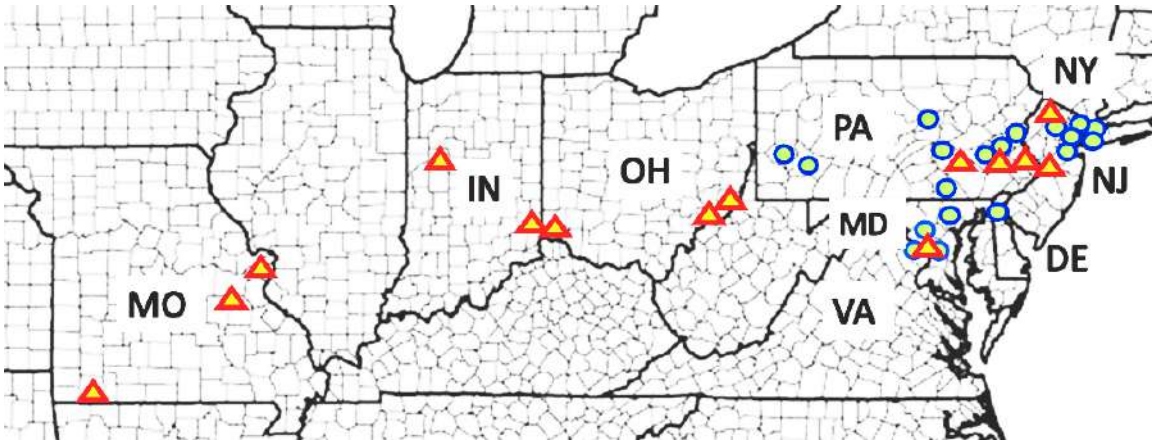

Figure 1. Updated, extended range of *Photinus scintillans*. Green circles are historical sites (Green 1956, Lloyd 1966). Red triangles show new and recently reconfirmed sites from this study and from personal communications of Lloyd, Stanger-Hall, and Lower.

Figure 2A–C. Study sites: A. Monroe County, Ohio, *P. scintillans* study site (photo: Hughes); B. Washington County, Ohio, *P. scintillans* study site (photo: Hughes); C. Franklin County, Indiana, *P. scintillans* study site in the winter, showing the steep grade. Photo by Meyers.

Field conditions. In the field, we found that any disturbance (such as catching specimens or obvious movements on our part) appeared to negatively affect the numbers of nearby displaying males; therefore, we minimized disturbance of the fireflies by sitting quietly in the forested sites with our voice recorders in order to record accurate native behaviors. We caught and dissected as few males as possible (<35 total) to obtain voucher specimens, and enough to confirm at each site that we had the correct species. After observations and data entry, all females (except the two we kept for oviposition studies) were released at the site where they were collected.

Figure 3. Female and male *P. scintillans* in stage 2 copulation. Photo by Hughes.

At our three study sites in Indiana and Ohio (Fig. 2A–C), plus the additional Hamilton Co., Ohio, site in 2019, we spent 34 nights over the course of the rise and fall of *P. scintillans* season (June and July) across four years (2016–2019) observing their behavior and courtship. We observed hundreds of flashing male *P. scintillans*, 15 perched females, and 10 copulation events (Fig. 3), seven of which we were able to time and closely observe. During peak nights, we observed >50 males displaying at once. Early- and late-season male flight densities were lower, with 2–10 males in the same areas. Sympatric and considerably larger *P. pyralis* fireflies often flew at the same time, but in more open areas adjacent to the forest sites of *P. scintillans*. The double-flashing *P. macdermotti* Lloyd males, also larger, were occasionally seen flying with *P. scintillans* at the Washington Co., Ohio, site (Fig. 4A). At the same site, *Photinus curtatus* (Fig. 4B) flew in low numbers (≈30% of *P. scintillans)* along the

Figure 4A, B. A: Two *P. scintillans* (left, center) are smaller than sympatric *P. macdermotti* (right; photo by Hughes). B: Often confused in the field and in museum collections, gradations of Division I *Photinus curtatus* (left and center) and Division 2 *P. marginellus* (right) sometimes fly with *P. scintillans* (not shown). Top row shows respective aedeagi (photos by Faust, Hughes, and Gilbert).

road margins, only occasionally mixing with *P. scintillans*, which were found in much higher unmixed numbers deeper in the forest. At the Franklin Co., Indiana, sites, several *Photuris versicolor* (Fabricius) group species displayed in trees close to the *P. scintillans* site.

General flight and courtship timing. *Photinus scintillans,* regardless of site, most frequently displayed in the woods, seldom flying beyond the tree canopy line of the forest. Males and females in the darker areas of the forest became active sooner than those displaying at the forest edge (ranging from 88 min to 35 min before sunset). As early as 30 minutes before sunset (dusk), brachypterous females (Fig. 5A) were observed to climb to more exposed perches on the leaf litter or low branches on the ground vegetation (usually ≈15cm above the ground) as males began to display low over the forest floor. Coupling usually began near the time of actual sunset regardless of male density. Male display declined sharply or ceased entirely by 50 minutes after sunset.

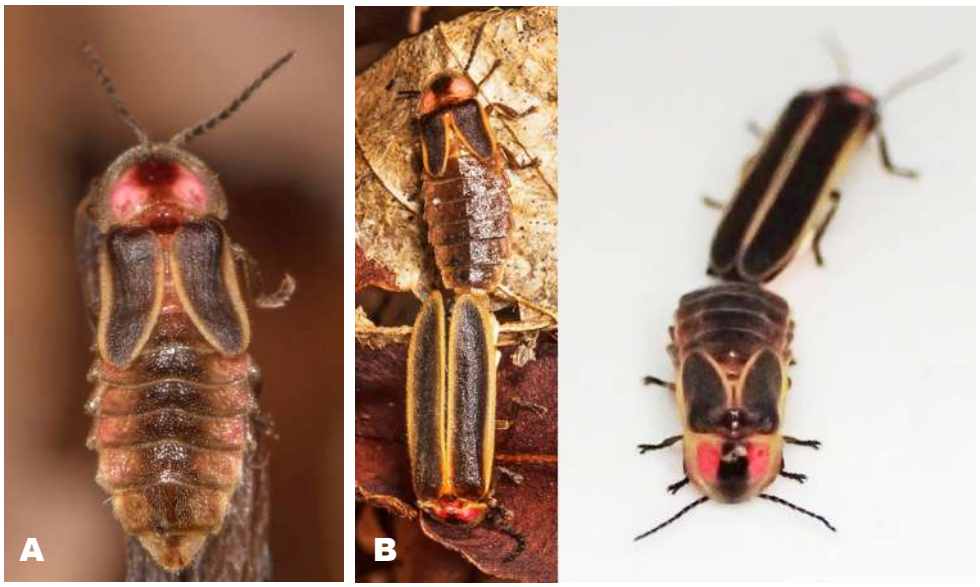

Figure 5A, B. A. Brachypterous female *P. scintillans* climb to their display perch at dusk. B. Coupling (stage 2 shown here) in *P. scintillans* begins at late dusk or dark on leaf litter or low perch and lasts ≈45+ minutes (photos by Hughes).

Nights of maximum competition. *Photinus scintillans* males flashed for one to two hours every night. Depending on male population density and cloud and forest cover, the onset of male courtship flights (flashing) varied. When moderate numbers of males were present, male flashing typically started an hour before sunset; however, when male numbers were very low at season's beginning or end, flashing started near the local time of sunset. For example, on June 17, 2017, a maximum peak night with over 50 males displaying at the West Harrison, Indiana, site, flashing began at 7:40 EDT, 88 minutes before local sunset, with low numbers of males flashing first in the darker portions of the forested hillside. By 68 minutes before sunset, large numbers of males were displaying. By 26 minutes before sunset, many females were climbing perches to begin courting. By sunset to 8 minutes after sunset, many couples had quickly swiveled from stage 1 (Suppl. video 1) to stage 2 (Fig. 5B), mating on the ground while other males continued to flash while flying low over the leaf litter and ground vegetation. By 9:45 or 37 minutes after sunset, a light drizzle began and all male flashing had ceased, though coupling continued in the leaf litter.

Display and courtship behavior. During mate search, *P. scintillans* males flew low (<45 cm), often just above the ground vegetation, while rhythmically emitting a single quick flash (≈0.15 sec. in duration) every ≈2 sec. Males travelled ≈15–45 cm horizontally per flash pattern, dipping slightly lower during the actual flash and rising slightly during the dark phase. At all three sites, we timed 27 males giving 252 flashes every 2.2 ± 0.35 sec (at temperatures of 23–27°C). If circling a potential mate or landing on vegetation, males would sometimes skip or compress a flash, but in general, the flash pattern was maintained.

During periods of high density, many of the lanterns of both males and females appeared orange-pink (Fig. 6A) instead of the more typical yellow observed during the resting state (Fig. 6B, C). The perched displaying females often twisted their abdomens while responding with a quick single or double flash immediately after the courtship flash of a passing male (Fig. 7). After a female response, the male circled and began to change the rhythmic species-specific timing of his courtship flash by flashing more often and less rhythmically, specifically towards the responding female. The male would land near (≈10–20 cm), but never on, the female. Soon the couple switched to a dialogue of arrhythmic single back-and-forth flashes and even ≈1 sec glows

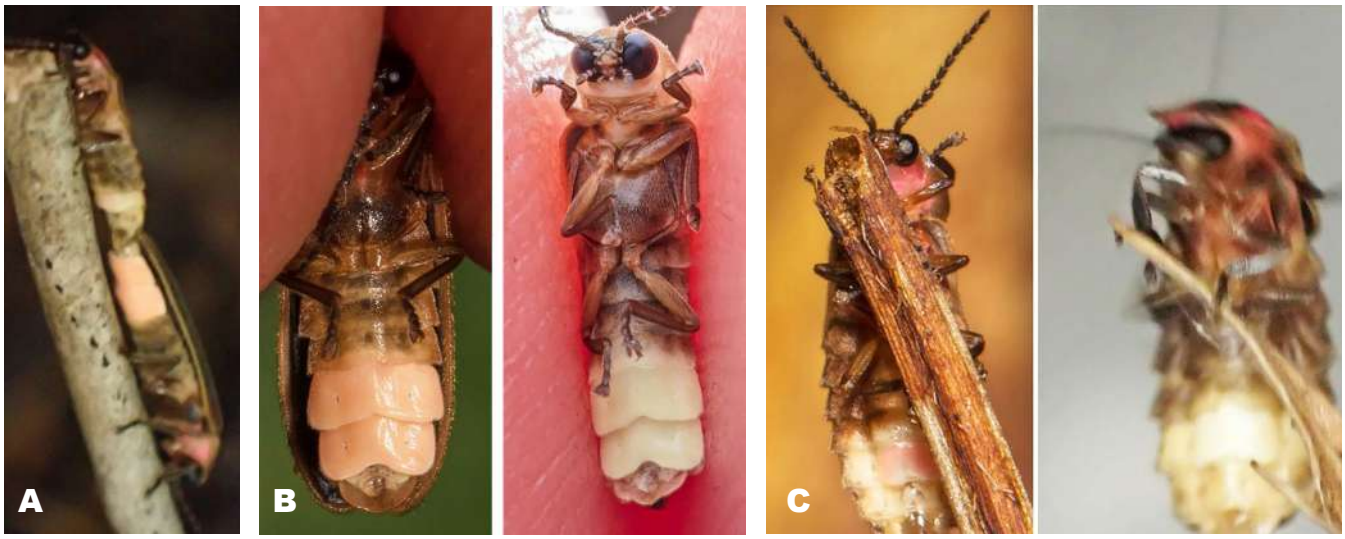

Figure 6A–C. A. Orange-pink coloration of both male and female *P. scintillans* lanterns can occur at times of courtship and mating. B. The often orange-pink coloration of lanterns of male *P. scintillans* evident during courtship displays (left) changes to the normal yellow lantern (right) in <5 minutes. C. First record that the lanterns of *P. scintillans* females also turn orange-pink in times of high motivation (left) before reverting to the more typical yellow coloration (right). Photos by Hughes.

(see Suppl. video 2). It was common to see one or two males courting the same female, but up to three males were observed simultaneously courting the same female, with two already landed and another still circling and flashing on the wing. The female did not answer every flash of every male, yet remained actively engaged in the courtship, often twisting and aiming her lantern while signaling toward the flashing males. After landing, males began searching among the vegetation for the exact location of the perched female, with all participants continuing to single-flash less rhythmically than when flying. From our observations, the first male to actually reach the female was usually the one to mate with the female. Stage 1 (Wing 1984, 1985; Lewis and Wang 1991) was brief, often lasting less than 1 minute. Stage 2 was considerably longer, with average copulation lasting 63 ± 21 min (n=7) for the duration of the copulation. Flashing ceased once stage 2 was achieved, and the orange tint of the lanterns reverted to the default pale yellow in minutes. Competing males dispersed once stage 2 was underway.

Figure 7. *Photinus scintillans* females twist their abdomens to better aim their response. Photos by Hughes.

Captive behaviors. A captive female was observed signaling while waving her abdomen during the night after capture, even in the absence of any males. This occurred at the same time *P. scintillans* were beginning their courtship displays in the wild. We also witnessed the lanterns of captive males in artificial surroundings turning pink-orange at the nightly time of flight. At the time at which nightly displays would begin in the wild, one male in captivity (kept at natural photoperiod and conditions) began to display pink-orange light organs as he approached and coupled with a captive female, with no courtship flight or flashing involved. The pink color of the lanterns faded back to the more typical yellow in under five minutes (n=3).

Adult morphology. Male body length (measured from anterior tip of pronotum to posterior elytral tips) averaged 8.7 ± 0.83 mm (n=20; Fig 8A). Brachypterous females averaged 7.5 ± 0.9 mm (n=4; Fig. 8B). All specimens measured ≈3 mm across at their widest point, measured abdominally between tergites 2 and 3. We observed geographic variation in body color: eastern Ohio *P. scintillans* with pale yellow or light gray abdomens were more typical in coloration to the historic populations near eastern Pennsylvania described by Say (1825) and Green (1956), while Indiana specimens tended to be darker, both abdominally and with darker pronotal markings (Fig. 9; also as illustrated in Faust 2017). There were exceptions at all sites, with some darker and others paler in both sexes. Dissection of males from all our sites revealed typical Green's Division II *P. scintillans* aedeagi (Fig. 10). Each male had two well-formed spermatophore-producing spiral glands (Fig. 11).

Figure 8A, B. A. Habitus of typical 7–9 mm male *P. scintillans*. B. Typical shortened elytra of flightless, brachypterous 7–9 mm *P. scintillans* female. Photos by Hughes.

Figure 9. More typical, paler yellow color phase Pennsylvania *P. scintillans* male (left) and darker Indiana *P. scintillans* male (right). Photos by Faust and Zloba.

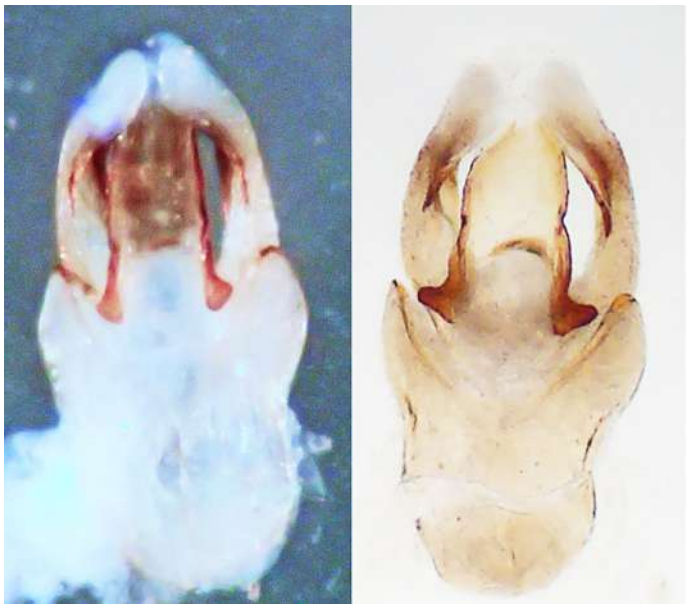

Figure 10. Green's *P. scintillans* Division II aedeagi: Ohio (left); Indiana (right). Photos by Faust and Hughes.

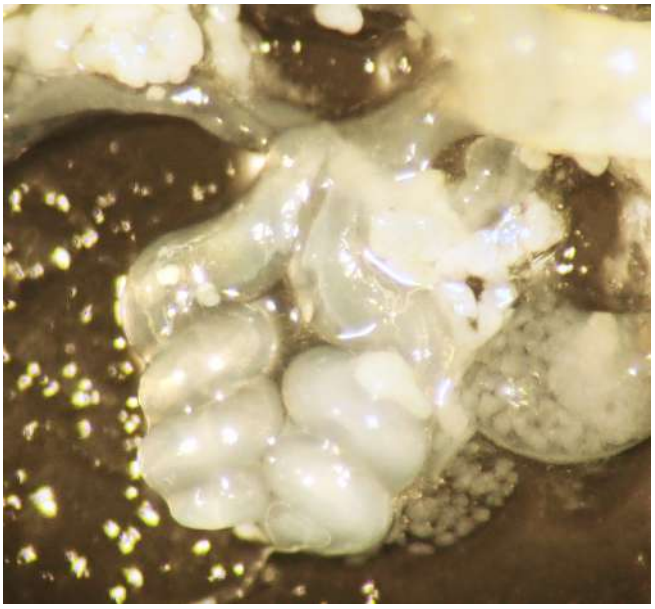

Figure 11. Double spiral glands of *P. scintillans* suggest that males have well-developed spermatophores, despite flightless females. Photo by Hughes.

Oviposition, eggs, and larvae. The two females used in oviposition studies were caught in the wild; thus, no prior mating history was known. One female was copulating upon capture. The second female copulated the next night in captivity. Observed captive copulation for the Ohio female was June 9, 2018 and her first eggs were noted two days later, on June 11. The Indiana female's final copulation was June 23, and she began ovipositing four days later, on June 27. Eggs were singly placed by the mother over a period of 1–4 days on native soil, a dried leaf, moss, or an apple slice placed in the container. The majority of eggs were laid on strands of moss (Fig 12A). These two clutches of eggs, from one Indiana female and one eastern Ohio female, numbered 9 and 11 respectively. Eggs were slightly oblong, pale, and smooth, yet slightly sticky; average egg size was 0.71 x 0.76 mm (n=5). No maternal care was noted. Females died within 24 hours after oviposition was completed. The clutch of 9 eggs from Indiana, kept in constant lab conditions with the lid seldom removed, succumbed to fungus after two weeks (Fig. 12B). The 11 eggs from Washington Co., Ohio, checked daily and kept in the shade (yet receiving natural photoperiod, temperature, humidity) had a 73% hatch rate. This hatch of eight eggs from the eastern Ohio female occurred ≈22 days after the first egg was oviposited. (As described in Fallon et al. (2018), *Photinus* eggs and larvae are notoriously difficult to rear.)

Just prior to hatch, one larva's jaws could be seen moving inside the thin egg membrane (Fig. 13A). By the time larval eclosion was complete, which took only 5 minutes, no remaining egg covering could be found near this newly emerged larva. The 2.5 mm larvae had brown eye spots visible and were initially white, darkening to pale grayish tan by the next day; they were covered with short bristles and showed the three pale longitudinal dorsal lines typical of *Photinus* (Fig. 13B).

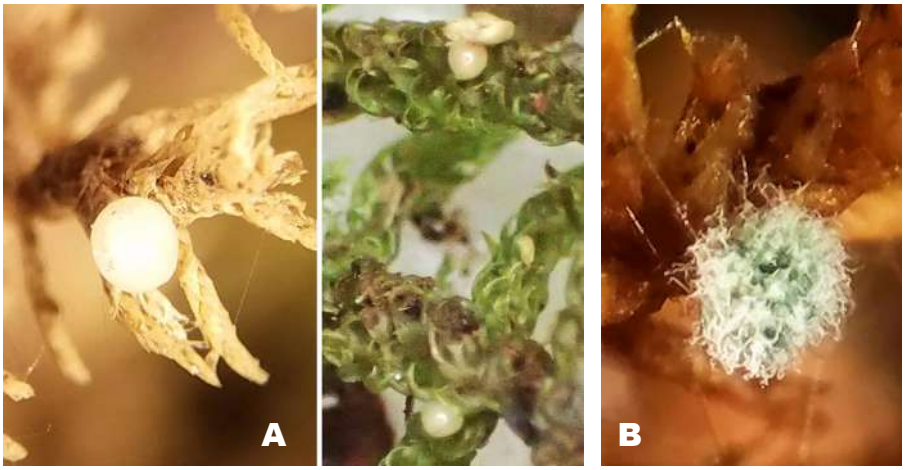

Figure 12A, B. A. Eggs were placed singly over 1–3 days on moss or soil. No maternal guarding occurred (photos by Zloba and Hughes). B. Some eggs succumbed to fungus (photo by Zloba).

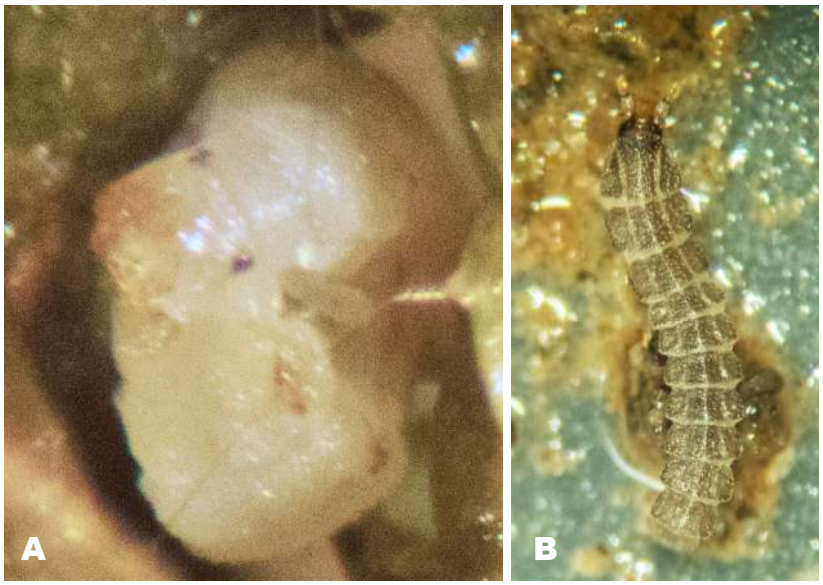

Figure 13A, B. A. After ≈22 days, larvae used their jaws to escape or eat the shell membrane. B. Two-day-old *P. scintillans* larvae darken from white on day 1 to grayish-brown by day 2. Photos by Hughes.

DNA. To further assure that our specimens were *P. scintillans* and not *P. punctulatus* Leconte, which is similar in flash pattern and appearance and also a Division II *Photinus* occurring in Illinois, Missouri, and elsewhere in the midwest, Farrington conducted DNA analysis. Based on GenBank data, all three of the specimens (two from Indiana, one from Ohio) genetically analyzed matched previously published *Photinus scintillans* COI DNA sequences with 94–98% similarity. The next most similar sequences returned in GenBank searches were *P. punctulatus* at 87% similarity.

Phoresy, parasitic, and predation observations of *P. scintillans*. At sunset on June 11, 2018 at the Washington Co., Ohio, site, a female *P. scintillans* was observed with an elongate springtail, *Entomobrya unostrigata* Stach (Katz et al.), on her tergites. The *P. scintillans* had just completed her nightly climb from the forest floor to her display perch (Fig. 14). The proximity of the photographer and her camera, combined with multiple camera flashes, alarmed the female *P. scintillans* and the ≈2mm

Figure 14. Phoresy of springtail on female *P. scintillans* (circled, top and bottom left). The photography caused the female to retreat, at which time the springtail disengaged. Note injured area, which may have served as a "handhold" for the springtail (circled in bottom right). Photos by Hughes.

springtail; the firefly retreated under a leaf, at which time the springtail detached and escaped. Multiple orange-brown mites were noted on an Ohio male and a single mite on a displaying female (Lloyd 1973; Fig. 15A). Though we did not maintain long-term captured *P. scintillans* specimens for phorid emergence studies, the abrupt overnight death and purple-tinged lanterns typical of phorid-infected fireflies were noted in one male (Fig. 15B).

Figure 15A, B. A. Mites (circled in red) were occasionally found on *P. scintillans* adults. B. An abrupt death and purple-tinged lanterns are typical of phorid-infected fireflies. Photos by Hughes.

On June 22, 2019, three predation events were observed at the Indiana site. After the main male display had ceased for the evening, a female of the *Photuris versicolor* group was seen feeding on a *P. scintillans* male (Suppl. video 3). A harvestman (*Leiobunum* sp.) and a spider (*Agelenopsis* sp.) also captured *P. scintillans* males (Suppl. Video 4). The *P. scintillans* gave occasional distress flashes while being fed on by the spider.

Habitat details. The two eastern Ohio sites were ≈345 km from the farthest eastern Indiana site (Fig. 1) and differed in habitat (Table 1). All sites occurred on relatively steep, secondary succession (from early to maturing in age) forested slopes facing from northwest to northeast. Elevations ranged from 212–305 m. Study areas of all three primary sites covered 0.25–0.75 hectares. The new 2019 Hamilton Co., Ohio, site was a similar habitat to the eastern Ohio sites. Despite similar annual rainfall averages (NRCC 2018), the Indiana site (Fig. 2C) appeared generally drier, with less lush ground cover than the richer, more mesic forest at the Ohio sites (Fig. 2A, B), though all sites would fall within the mixed mesophytic forest type (Table 1).

The Franklin Co., Indiana, site, at 295 m, facing north to northwest, was ≈900 m from the Whitewater River, with a small wet-weather stream within 100 m (Fig. 2C). Formerly an old field, this wooded hillside has trees that are primarily 20- to 45-year-old volunteer species (see Table 1) with sparse herbaceous ground cover. This site is bisected by Interior Plateau ecoregion III (northern bluegrass subdivision IV) and Eastern Corn Belt Plains ecoregion III (loamy high lime till plains subdivision IV; Omernik 1987, U.S. Environmental Protection Agency 2018). The topography/substrate consists of steep, sloping clayey and silty soil types of the Eden-Carmel and Miami-Xenia-Russell associations, intermixed with Ordovician limestone (Rogers et al.1950).

The Monroe Co., Ohio, site, at 214 m, is a forested slope facing northeast with all-year flowing Narrows Run less than 33 m (108 ft) away, which flows into the nearby Ohio River (Fig. 2A). A smaller seasonal-flowing headwater stream is <10 m from the display site. Like the Washington Co., Ohio, site below, the floor of this verdant 100-year-old forest is covered with herbaceous plants (Table 1).

The Washington Co., Ohio, site, at 212 m, faces northwest, with the Little Muskingum River ≈150 m to the west and a seasonal headwater stream within 15 m from the display site (Fig. 2B). This forest was selectively cut 25 years ago, but retains a maturing forest profile. The forest composition and ground cover are similar to the Monroe Co., Ohio, site (Table 1). These two eastern Ohio sites are both in the Permian Hills (ecoregion IV) of the Western Allegheny Plateau ecoregion III (Omernik 1987,

US Environmental Protection Agency 2018). Predominant soils in both counties are in the Gilpin-Upshur-Lowell-Guernsey series, with moderately deep, well drained silty-loamy soils underlain by clay (Hayhurst et al. 1974, Ohio Department of Agriculture, Soil Regions of Ohio 2018).

Seasonality with range of dates (with mGDD). Over the four-year period of 2016–2019 at our study sites, *P. scintillans* were observed for roughly four to six weeks, June 7–July 22. Each season, male displays began with low numbers in early June, built to peak numbers by the third week of June, and returned to diminishing or absent display by season's end in mid- to late July. The overall Fahrenheit degree-day range was 848 to 1890 mGDD (474 to 1058 mGDDC), with no adults present after 1950 mGDD (1092 mGDDC). At this 39°N latitude, peak numbers of *P. scintillans* clustered around the week surrounding June 20 or ≈1200–1400 mGDD (672–784 mGDDC).

The geographic outliers collected by Dr. Kathrin Stanger-Hall used in our range extension map and analyzed in Stanger-Hall and Lloyd (2015), were collected from May 31–July 15: June 1, 2003 in Barry Co., Missouri (1044 mGDD and 585 mGDDC); on July 15, 2002 in Tippecanoe Co., Indiana (1689 mGDD and 946 mGDDC); and in the epicenter of historic *P. scintillans* range, May 31, 2010 in Bucks Co., Pennsylvania (803 mGDD and 450 mGDDC; Stanger-Hall, personal communication).

Additional Ohio and regional Lampyridae-related species records and observations. During this study, additional records and unique observations include reports of populations of the popular discontinuously synchronous-flashing *Photinus carolinus* Green (Moiseff and Copeland 1995; Faust 2010) in southeastern Ohio in Adams, Scioto, and Monroe counties, including one distinct Monroe Co. population that flies only late at night, after midnight. Far to the north, Cleveland Metroparks naturalist Carly Martin confirmed a robust population of *P. carolinus* displaying in Cuyahoga Co. at South Chagrin Reservation on June 29, 2019 just southeast of Cleveland. A new state record of *Photinus cooki* Green was found in Clermont Co., with one normally lanternless male having atypical yet near-complete lantern tissue in four circular areas on segments 6 and 7 (Fig. 16). A new state record of *Photinus australis* was collected at Cincinnati Nature Center in Clermont Co., Ohio; upon dissection, males were found to have double spermatophore spiral glands (Fig. 17), similar in appearance to *P. scintillans* yet more robust. A new state record of *Photinus acuminatus*, the first example found in decades, was found in Adams or Scioto Co., Ohio, on July 9, 2017 (Fig. 18), nearly 436 km from the nearest record (Pisgah Mountain, North Carolina; Lloyd 1966). Two *Pleotomus davisii* Leconte larvae were discovered in Adams Co., and one was kept for observation from June 30–Aug 1, 2018 (Fig. 19).

According to our dissections and Lloyd (1967), both *Photinus curtatus* and *P. marginellus* are found in Ohio. *Photinus curtatus* were abundant in proper habitat in Adams, Monroe, and Washington counties in Ohio, while *P. marginellus* were present in Licking, Hocking, and Geauga counties in central and northern Ohio (authors' personal observations; Fig. 4B).

Figure 16. Ohio state record for typically lanternless *P. cooki*. This male had unusual partial lantern tissue on segments 6 and 7. Photo by Faust.

Figure 17. Another Ohio state record, *P. australis*, has large spermatophore-forming spiral glands. Photo by Faust.

Figure 18. Extremely rare *Photinus acuminatus* was found in southern Ohio for the first time anywhere in decades, 436 km north from where the next closest record was recorded. The distinct aedeagus (left) and larger male *P. acuminatus* (left) is next to two much more common *P. curtatus* (right). Photos by Hughes.

Figure 19. We found and kept a seldom-seen *Pleotomus davisii* larva for two months. Photos by Hughes.

In Monroe Co., Ohio, *Pyractomena borealis* larvae were repeatedly observed sipping maple sap at the sap flows in late winter and early spring (Fig. 20). In Adams Co., Ohio a female *femme fatale Photuris versicolor* group female was observed for three nights hunting at dusk within a node of displaying *P. pyralis* males beside small stream in the woods alongside an open area. With her flashes and behavior, she mimicked both male and female *P. pyralis* according to her position. When perched, she would often flash at the courting *P. pyralis* males using the proper female *P. pyralis* response delay. In flight, this female *Photuris* was dipping while flashing to mimic the flight posture of *P. pyralis* courting males. We did not see her capture any *P. pyralis*, but we did see her dart in flight at *P. pyralis* males several times in an unsuccessful aerial predation attempt.

Figure 20. *Pyractomena borealis* larvae were found at maple sap flows in Monroe Co., Ohio. Photo by Hughes.

Though our five-year, multi-county search for *Phausis reticulata* Say and *Phausis inaccensa* Fender populations in Ohio continues without success (De Cock et al. 2014, Faust and Forrest 2017), we did find two robust extant populations of *P. reticulata* in Indiana and Kentucky, far from the currently accepted range in the southern Appalachians; both populations are within 18 km of each side of the Ohio River (Fig. 21). Naturalist Linda Romine, aware of our *Phausis* search, contacted us about a population she had discovered at Saddle Lake Recreation Area in Perry Co., Indiana, on May 18, 2019, 214 km southwest of Cincinnati; this site was visited again on May 21 by Romine and Hughes. Dr. Rob Naczi, who also knew that we were looking for *Phausis*, remembered his collection of *P. reticulata* from 20 years earlier at the northern Kentucky site and provided us with photos and location details that we used with success; the second population of *P. reticulata* was confirmed by Zloba on June 23, 2019 at Curtis Gates Lloyd Wildlife Management Area in Grant County, Kentucky, just across the Ohio River, 35 km from Cincinnati (Fig. 22; Suppl. Video 5).

Finally, with the help of Max Henschen and Chris Fox (Indiana) and Christina Feng and Joseph Nelson (Illinois), we confirmed two additional sites for the newly described firefly *Photuris walldoxeyi* (Faust and Davis 2019) in Indiana (Beanblossom Bottoms Nature Preserve) and Illinois (Cache River and Heron Pond State Natural Area).

Figure 21. *Phausis reticulata* were found in Perry Co., Indiana, and Grant Co., Kentucky, far from their accepted range. Indiana male (left); 4 glow-spot Kentucky female (right). Photos by Hughes and (bottom right) Myres/Zloba.

Figure 22. Grant Co., Kentucky: *Phausis reticulata* coupling with apterous females takes place in <10 minutes, as opposed to *Photinus scintillans* with brachypterous females, which couple for nearly an hour. Photo by Zloba.

Discussion

Range. Results of this study combined with unpublished record details by Stanger-Hall, Lloyd, and Lower (personal communication) have tripled (from Lloyd's 1966 map) the east–west range of *P. scintillans*, from New York to southwestern Missouri. The north–south distribution remains relatively unchanged and narrow across its entire range, similar to the earlier map. Many questions about the range of *P. scintillans* remain: could the last glacial maximum have influenced this narrow band-like distribution? Did the ancient Teays River drainage play a role? Why does this species appear to have a southern limit? Could maximum summer temperatures be a limiting factor? The presence of *P. scintillans* at the farthest west location in Roaring River, Missouri, remains a question to be further explored: is this the farthest west population? It now appears, however, that the Indiana *P. scintillans* are well established in both the southeast and northwest parts of the state and probably elsewhere. We suggest that our eastern Indiana *P. scintillans,* observed in high numbers for four seasons, are not nursery stock escapees, as Lloyd initially suspected for his northwestern Indiana population (personal communication), but are instead robust established populations. The wide habitat variations of the known populations (including pine barrens, mesic Appalachian forests, more xeric forested hillsides in the plains region, and riverine environments in the Midwest) suggest this species is not confined to any one specific habitat, as was suspected for the past 60 years (Lloyd 1966), though wooded areas and proximity to a river are common to all our study sites. Just as this study reflects the cooperation and engagement of multiple citizen scientists, this new range extension map represents the collaboration and sharing of data of six researchers (see acknowledgements) spanning 53 years on multiple unrelated surveys (Lloyd 1966; Stanger-Hall, Lloyd and Lower, personal communication), with each hoping to better understand *P. scintillans*. We believe that many additional populations of *P. scintillans* will be located, with the most likely new populations being in the states already discussed. Additionally, Illinois, Kentucky, West Virginia, and other states likely harbor populations waiting to be discovered.

Copulation times. The finding that *P. scintillans* males have double spiral glands, implying the production of spermatophores (van der Reijden et al. 1997, Lewis et al. 2004, South et al. 2010), and more prolonged mating times of nearly an hour differ from earlier studies conducted on *Photinus collustrans* Leconte, also with brachypterous females. Wing (1984, 1985) compared the mating times and behaviors of *P. macdermotti,* a species with alate females, to *P. collustrans*, a species with brachypterous

females. Though both species used the stage 1 and stage 2 copulation positions (Wing 1985, Lewis and Wang 1991), Wing found great differences in mating times, with the winged females having much longer copulations (1–9 hours) than the brachypterous females (≈1 minute), which lived in burrows. Wing's *P. collustrans* males appeared to lack developed accessory glands, whereas *P. scintillans* males have well-developed spiral glands. Could the simple fact that *P. collustrans* females display from or near their burrows, whereas *P. scintillans* females climb to a display perch (similar to species with alate females), provide an unrecognized evolutionary driver to male competition, development, and copulation duration and behavior? Could *P. scintillans* represent an intermediate evolutionary step in reproductive structures and behaviors between Division II *Photinus* species with fully alate females and those with more neotenic females (Cicero 1988), such as *P. collustrans*? The *P. scintillans* brachypterous females did not exhibit maternal care and died soon after oviposition, unlike the totally apterous females that show maternal care of the egg clutch in some *Phausis* species (De Cock et al. 2014, Faust and Forrest 2017).

Density and display times. The higher population density of males appears to drive the males to begin flashing earlier relative to sunset as compared to nights of less competition. Lloyd (1966) alluded to this same observation for *P. scintillans* that we observed in our studies. This phenomena of greater numbers of males within a given area (and resulting higher competition) driving an earlier start of display relative to sunset has been noted in males of *P. carolinus* (Faust 2010, 2017), *P. sabulosus* (LFF unpublished data 2018), *P. curtatus and P. pyralis* (per obs.), *Photuris walldoxeyi* Faust (Faust and Davis 2019), and *Photuris frontalis* LeConte (LFF unpublished data). We noted that males landed near but never directly on the females, similar to the landing distances reported for *Photinus carolinus* (Copeland et al. 2008). Our flash timings agree with Lloyd's (1966) and Stanger-Hall and Lloyd's (2015) descriptions, though all three sites of our Ohio and Indiana sites were warmer, causing the *P. scintillans* males to give slightly quicker intervals. In 2016 at the Indiana site, before detailed timed studies began, it was informally noted that when temperatures were over 30°C following an unusually hot day, the flashes appeared to occur almost every second.

Flash color. The orange-pink-tinted quick flashes caused us to initially believe we had found a *Pyractomena* sp., so we caught the first four specimens and were surprised to find they were all small *Photinus*. The size and quick flashing, combined with the overall darker coloration of these Indiana *P. scintillans,* caused us to be uncertain whether we had found a *P. punctulatus* population or a new *P. scintillans* population (Faust 2017). Our subsequent discovery of additional populations in eastern Ohio, DNA verification, and the presence of brachypterous females confirmed our original conclusion that these new eastern Indiana fireflies were *P. scintillans.* The existing common names provided in Faust (2017) of "Pine Barrens firefly" (Lloyd) and "Pale" (traditional) or "Yellow-Bellies" (Faust) are not a good fit in these western populations, with their darker coloration and different habitats. We suggest **"**Low Pink Winkers" as an additional informal name because the males fly very low, just over the forest ground vegetation, and flash very quickly ("winking") in yellow or pink-orange.

Though the phenomenon of displaying pink-orange lanterns (instead of yellow) is especially noticeable and frequent in *P. scintillans*, we also want to note that we have observed and documented this characteristic (Faust 2017) to a lesser degree in other *Photinus* species, both Division I and Division II (Green 1956), at times of high densities and competition: *Photinus australis*, *P. consanguineus* group "Cajun single-flasher" and *P. macdermotti*, *P. knulli*, and *P. sabulosus*. *Photinus pyralis*, *P. consimilis*, and *P. carolinus* (Faust 2010) have also been seen glowing orange-pink, although the reasons may differ. Interestingly, the great fireflyer Frank McDermott (1911) reported this same orange color in both *P. pyralis* and *P. scintillans* over 100 years ago. Pigment analysis studies by University of Georgia's Dr. Kathrin Stanger-Hall are looking into the mechanisms causing this color-changing phenomenon. We illustrate and report for the first time that *P. scintillans* females can also display this pink-orange lantern coloration at times of high receptivity. This unusual coloration rapidly fades (in <5 minutes) once the stimulus of courtship is removed.

Coloration. All three populations contained many but not all externally darker examples than the more typical, paler eastern Pennsylvania *P. scintillans*. Generally, the eastern Ohio *P. scintillans* were intermediate in coloration compared to specimens previously described by Green (1956), having pale yellow or light gray abdomens. The abdomen and pronotal markings of Indiana specimens tended to be darker. Awareness of these regional coloration differences is important in recognizing, discovering, and identifying new populations.

Uneven distribution of species. We have also noted over the years that when large numbers of one *Photinus* species fly at dusk in a certain area, other *Photinus* species that are regionally present in similar habitats are often absent or found in very low numbers, whereas without a competitive species, their numbers can be large. Do individual microhabitats or larval/adult competition favor the larvae of one *Photinus* species over another species, at least locally? In the past, we have observed this uneven distribution in population numbers in *Photinus scintillans*, *P. marginellus*, *P. curtatus*, *P. australis*, and *P. sabulosus* in Indiana, Ohio, Virginia, and Tennessee. As stated earlier, *P. marginellus* and *P. curtatus* have been considered the same species or different species (Green 1956, Lloyd 1967, Lloyd 2001) over the past 150 years. We found both populations present, though somewhat regionally separated, during our studies in Ohio (Fig. 4B).

Seasonality. In Faust (2017), the suggested degree-day ranges for *P. scintillans* are recorded as ≈1200–2000+ mGDD (672-1120+ mGDDC), taken from personal observations and museum specimen records. Though useful for general regional prediction purposes, the heuristic use of degree-days is especially valuable for the prediction of the rise and fall of a species' season for local populations that are followed and documented year after year. This paper suggests using an earlier calendar and degree-day start range for these Ohio and Indiana populations at 39°N (and geographic outlier and historic range additions from 36–41°N), with the greatest chance of successful searching for new populations happening at ≈800–1600 mGDD (448–896 mGDDC) from late May to early July, with peak numbers likely occurring in late June at ≈1200 mGDD (672 mGDDC).

Phoresy. We present an example of possible phoresy of an elongate springtail, *Entomobrya unostrigata*, on a lampyrid, a female *P. scintillans*. This is the first report of modern-day Collembola phoresy on an extant beetle, though this relationship has been documented in other beetle specimens found in amber (Penney et al. 2012, Grunemaier 2016). We suggest the possibility that springtails may climb on female *P. scintillans*, which hide in undergrowth during the day. The darker, injured area on this particular female firefly's latero-dorsal abdomen may have provided a good "handhold" for this particular springtail (Aron Katz, personal communication). The springtails perhaps also take advantage of the lack of firefly predation due to the chemical protection most fireflies possess (Eisner et al. 1978). The female *P. scintillans* ascend to perches each evening at sunset for courtship. The males land and mate with these females, at which time the springtails could either transfer to the flighted males (also chemically protected) for dispersal or stay with the earthbound females.

Conclusion. Beginning with a three-day firefly workshop taught by Faust in 2016 as part of the Advanced Naturalist series at Cincinnati Museum Center's Edge of Appalachia Preserve, a number of enthusiastic students "saw the light" and took their new knowledge into the field. From the initial Indiana discovery by Zloba just after the class in 2016, when he observed that the firefly display was "somehow different" from what we had observed in the past, followed by finding new *P. scintillans* populations in Ohio by Hughes in 2017 and 2018 and Zloba in 2019, we learn that when trained eyes begin to look, new truths are recognized that may have been hiding in plain sight all along. This same concept holds true for the possible phoretic springtail–firefly association we witnessed and the new state and regional records we report in this study. Once an observer knows enough to notice something different, and that an unexpected occurrence is indeed possible, new discoveries will be made. This study was a truly collaborative effort of the authors being helped tremendously by field volunteers, family, property and facility owners, and academic firefly (and springtail) researchers sharing their opinions and unpublished field notes from previous studies. We suspect that many additional *P. scintillans* populations are awaiting recognition across Ohio and the eastern United States.

Acknowledgements

We thank landowners Peggy Klingensmith (Indiana), Richard and Linda Stalder, Laura and David Hughes, John Howard (Ohio), and J.D. and Mike Verheeck (New Jersey); Paul J. Zloba and Lexi M. Zloba for help with initial Indiana observations; James Meyers for the Indiana habitat photo, Harmony Myres for *Phausis* female glow-spots; Aron Katz for the springtail identification; Kathrin Stanger-Hall for DNA and manuscript discussions and sharing details of outlier specimens; Jim Lloyd for historical and new site information and discussion; Sarah Lower for sharing several recent *P. scintillans* sites in the historic range region; Linda Romine, Sheila Cox Riley, and the Cincinnati Nature Center for Clermont Co., Ohio, collections and observations leading to new records; Linda Romine and Rob Naczi for leading us to new *Phausis* populations; Linda Gilbert, Carly Martin, and Cleveland Metroparks for Geauga Co., Ohio, data; Saddle Lake Recreation Area (USFS) in Perry Co., Indiana, and Curtis Gates Lloyd WMA (KYFW) in Grant Co., Kentucky; Max Henschen, along with Chris Fox of the Sycamore Land Trust in Indiana, and Christina Feng and Joseph Nelson of the Illinois Department of Natural Resources for initial observations; the Cincinnati Museum Center for DNA extractions and analysis at the Geier Center and the use of laboratory facilities at the Richard and Lucille Durrell Edge of Appalachia Preserve System; and Edge of Appalachia Preserve director Chris Bedel.

Supplemental Videos

Folder of all 5 supplemental videos available at: https://vimeo.com/user87539636/

Supplemental video 1 (https://vimeo.com/351270919). Swiveling by *P. scintillans* couple as they change from stage 1 to stage 2 of copulation. Video by Hughes.

Supplemental video 2 (https://vimeo.com/351270932). Three competing *P. scintillans* males court a perched female showing a variety of courtship flashes. Video by Hughes.

Supplemental video 3 (https://vimeo.com/351274504). A *femme fatale Photuris* eats a *P. scintillans* male. Video by Zloba.

Supplemental video 4 (https://vimeo.com/351273215). An *Agelenopsis sp.* spider and a *Leiobunum sp.* harvestman have captured and feast on two *P. scintillans* males. Video by Zloba.

Supplemental video 5 (https://vimeo.com/351270942). *Phausis reticulata* mate in Grant Co., Kentucky, which is just 35 km from Cincinnati. Video by Zloba.

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