Journal of Crustacean Biology Advance Access published 29 March 2019

The Crustacean Society

Journal of Crustacean Biology

Journal of Crustacean Biology 39(3) 227–243, 2019. doi:10.1093/jcbiol/ruz009

Luminaries of the reef: The history of luminescent ostracods and their courtship displays in the Caribbean

James G. Morin

Department of Ecology and Evolutionary Biology, Cornell University, Ithaca, NY 14850, USA

Correspondence: J.G. Morin: email: jgm18@cornell.edu

(Received 17 December 2018; accepted 15 February 2019)

ABSTRACT

About half of the known +300 species of cypridinid ostracods (Ostracoda, Myodocopida, Cypridinidae) (256 described and > 62 known but undescribed) are luminescent, probably all to deter predators, but also about half of the luminescent species use their light in courtship displays. These courtship displays have only been shown to occur in the Caribbean and nowhere else. The initial discovery of these courtship displays occurred in 1980. This publication provides 1) a summary of the life cycle and courtship mating system of bioluminescent signaling cypridinid ostracods, 2) a history of the systematics of the family, and 3) the initial discoveries of their luminescence and details of the luminescent system. Observations of luminescence in ostracods began off the coast of India in the mid-18th century and expanded to Japan and elsewhere, especially in the 20th century. Studies on the biochemistry and mechanisms of light production by E. Newton Harvey and his academic descendants, using the iconic Japanese ostracod, called *umi-hotaru* (*Vargula hilgendorfii* (G.W. Müller, 1890)), started in the early 20th century and continue today. Some of the conflicting semantic difficulties between systematists and biochemists are discussed.

Key Words: *Cypridina*, Cypridinidae, E.N. Harvey, cypridinid luciferin, cypridinid luciferase, Myodocopida, Umi-hotaru, *Vargula hilgendorfii*

EIGHTEENTH INTERNATIONAL SYMPOSIUM ON OSTRACODA

INITIAL DISCOVERIES OF LUMINESCENT COURTSHIP DISPLAYS IN CYPRIDINID OSTRACODS

It had long been postulated that the luminescence ejected from some myodocopid ostracods when disturbed functions to deter aggressors (Doflein, 1906; Dahlgren, 1916; Morin, 1983, 1986; Morin & Cohen, 1991). But my first field observations upon seeing organized rows of discrete points of blue light hanging in the water column above a Caribbean reef on a moonless night suggested that the bioluminescence from ostracods might be more complex than a mere spewing light upon irritation. These initial observations occurred on 10 June 1980 on a night dive on the back reef of Teague Bay on the north side of St. Croix, US Virgin Islands (ca. 17° 45.9′N, 64° 37.2′W) while I was diving with William D. McFarland. Shining a beam of light at a glowing spot revealed nothing. But by casting the light along the path of the spots revealed one or more tiny, rapidly swimming crustaceans, which I surmised were ostracods. On a subsequent night dive in the same area I managed to capture some individuals, which verified my presumption that they were cypridinid ostracods. This event marked the beginning of an expanding study into the complex life of ostracods that produce luminescent courtship signals. Knowing what we know now of the ubiquity of ostracod courtship displays in the Caribbean (Fig. 1), along with the rapid increase in scuba diving during the past 50 years, it is clear that someone was bound to discover these spectacular near-nightly displays. It just happened to be me.

But determining the courtship and reproductive details of how tiny, 2–3 mm crustaceans swimming rapidly in a big, dark ocean above reefs is not an easy feat. It has taken years of painstaking research, often advancing only as new technology has permitted. My initial two summers of research were spent with Biff Bermingham on the reefs of Teague Bay and Buck Island just north of St. Croix, during which we determined the basic diel, spatial, and courtship characteristics of the displays (Morin & Bermingham, 1980). From our studies we hypothesized that only the males produce the displays in order to attract and guide sexually receptive females, which can luminescence when disturbed, but not during the displays. My cartoon, circa 1982 (Fig. 2), used in seminars, encapsulates the basics of the hypothesis, which still continues to be supported without much change today (Gerrish

Figure 1. Luminescent courtship displays (lateral, vertical upwards, and vertical downward displays) of three cypridinid ostracod species at night over a shallow back reef near South Water Cay, Belize. Stitched images taken by Martin Dohrn (Ammonite Films) by beam-splitting the image to both a low-light-level camera, which shows the luminescence, and an infrared camera done in conjunction with an infrared light, which shows the background reef, and then superimposing the two (used with permission). This figure is available in color at *Journal of Crustacean Biology* online.

Figure 2. Cartoon of a male cypridinid ostracod emitting a luminescent coded message [the Morse code message spells: T R Y M E] seeking to attract a receptive female observing nearby (by J.G. Morin, *circa* 1982). This figure is available in color at *Journal of Crustacean Biology* online.

& Morin, 2016). When sufficient ambient darkness is achieved, males leave the benthos, enter the water column and repeatedly secrete rows of light pulses seeking to attract sexually receptive females, which target the light trail without luminescing, and swim to the male, whereupon they copulate with internal fertilization (Morin & Cohen, 2010; Rivers & Morin, 2013). The female then returns to the benthos and broods her fertilized eggs for the next few weeks (Gerrish & Morin, 2008), while the male continues his nightly quest, using his ritualized displays in attempts to attract and inseminate other receptive females.

While luminescent cypridinid ostracods occur in all oceans from polar to tropical waters and at virtually all depths (surface to > 4,000m), only the Caribbean has one monophyletic clade of more than 87 cypridinid species, with one known exception, which has been found to also produce extraordinarily complex sexual courtship displays involving the ejection of pulses of light into the sea in near darkness (Morin, 1986; Morin & Cohen, 1991; Gerrish *et al.*, 2009). Depending on the species, males produce their displays above specific microhabitats within reefs and seagrass beds (Morin & Cohen, 1991, 2010; Gerrish & Morin, 2016). Displays occur throughout the year as ambient light conditions drop below a critical near-dark threshold each evening, after sunset or moonset, whichever occurs later (Gerrish *et al.*, 2009). The lone exception in the clade is a luminescent, but non-signaling species *Vargula tsujii* (Kornicker & Baker, 1977), which occurs along the Pacific coast of North America from northern Baja California to central California (Kornicker & Baker, 1977; Cohen & Morin, 1990, 2003). This peculiar distribution may represent a remnant of the signaling clade that was cut off during the rise of the Isthmus of Panama 3.1–2.8 mya (Coates & Obando, 1996; Lessios, 2008; O'Dea *et al.*, 2016) with subsequent loss of signaling by this species' ancestors (Cohen & Morin, 2003). Despite my many efforts to observe and collect in appropriate habitats and times, no signaling species have been found along the Pacific coast of the Isthmus of Panama or anywhere else in the world (personal observation).

Based on both morphological and molecular data, the signaling clade consists of a minimum of 87 species in three major subclades (Figs. 3, 4), which we currently refer to as the H-Group (Fig. 4A) (containing at least two undescribed genera and at least 35 species), T-Group (Fig. 4B) (containing *Kornickeria* Cohen & Morin, 1993 with at least 25 species), and the F-Group (Fig. 4C) (containing *Photeros* Cohen & Morin, 2010 with at least 19 species and *Enewton* Cohen & Morin, 2010 with one species) (Cohen & Morin, 1990, 2003; Torres & Gonzalez, 2007; Morin & Cohen, 2017). Two other clades (the C-Group with at least three species and the Z-Group with at least three species) also belong to this signaling clade, but their phylogenetic relationships are uncertain at this time (Cohen & Morin, 2003).

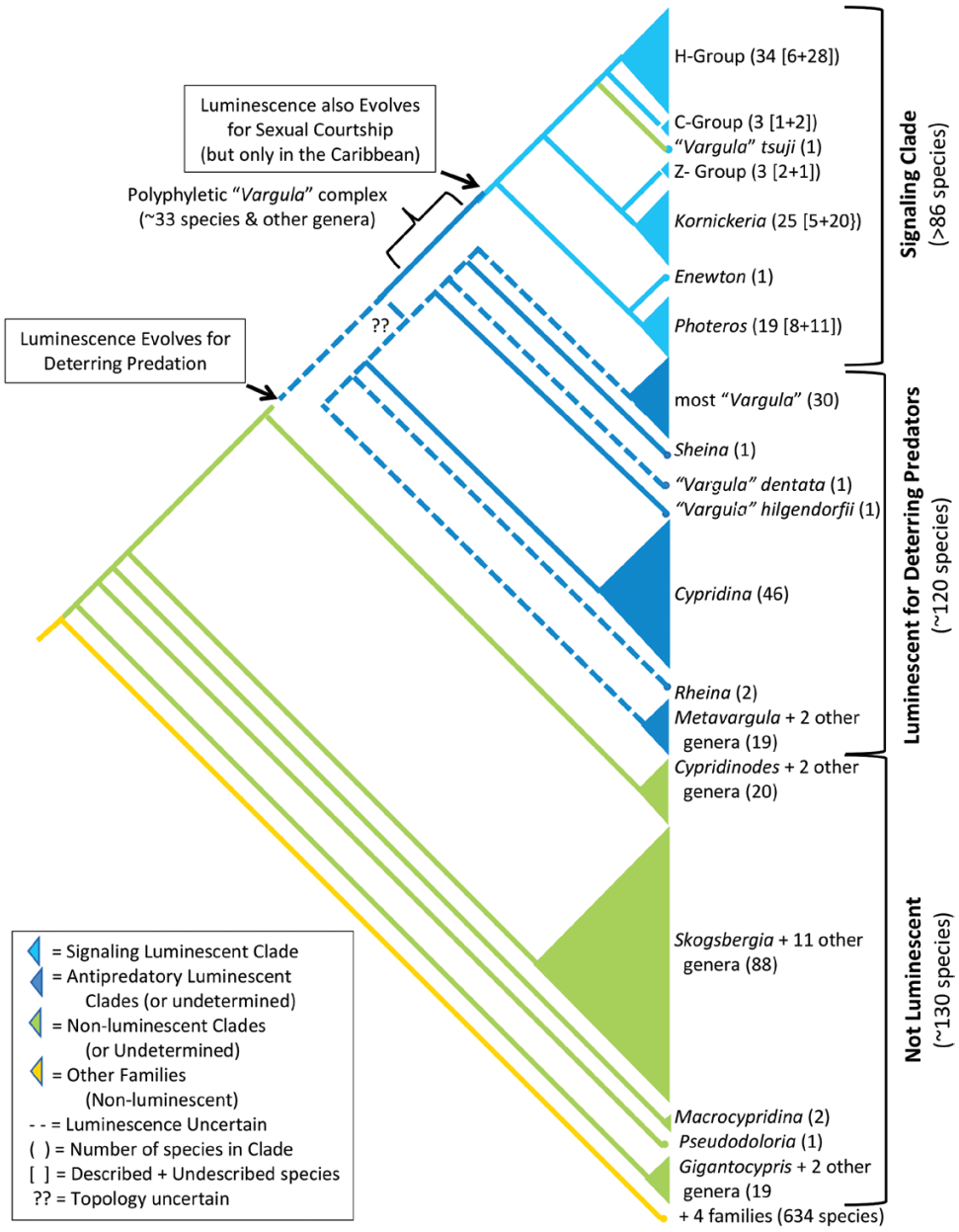

Figure 3. Phylogeny of Cypridinidae with respect to bioluminescence. About a third of the (basal) cypridinid species are non-luminescent, while nearly two-thirds produce light to deter predators. Of those that do luminesce, about a third to half have also secondarily evolved the ability by males to use their light in courtship displays [updated and modified from Cohen & Morin, 1990, 2003; Morin, 2011; cypridinid data entries in the World Ostracoda Database, World Register of Marine Species (WoRMS) [http://www.marinespecies.org/ostracoda/], and numerous field collections; see also Table 1]. The signaling clade numbers are based on both published genera and species and on field collections and notes pertaining to currently undescribed species. This figure is available in color at *Journal of Crustacean Biology* online.

The signaling species that have been described were all initially placed in *Vargula*, but based on more detailed morphological evidence they are all being reassigned to new genera including *Kornickeria*, *Photeros*, *Enewton*, and others to be described (Cohen & Morin, 1993, 2010; Morin & Cohen, 2017; N. Reda *et al.*, unpublished data). Our morphological analyses across the family (Cohen & Morin, 1990, 2003), have shown that *Vargula* has been a kind of "catch-all" genus (see below); many non-signaling luminescent species found outside the Caribbean are also currently included in *Vargula* and should be placed in still other undescribed genera (Fig. 3).

The displays themselves provide us with a kind of signal "fingerprint" that allows us to easily identify distinct species in the field (Morin & Cohen, 1991, 2010, 2017; Gerrish & Morin, 2016). Within the signaling clade, sexual selection for species-specific displays, along with restricted gene flow engendered by the limited dispersal capabilities of these ostracods, almost certainly has led to the evolution of a species flock with high diversity in the Caribbean.

LIFE CYCLE AND MATING SYSTEM OF BIOLUMINESCENT SIGNALING CYPRIDINID OSTRACODS FROM THE CARIBBEAN

This section summarizes 1) the salient features of the reproductive and life cycle patterns of the courtship clade (some of these

patterns occur in all or almost all members of the Cypridinidae, but vary especially in non-luminescent species), 2) the features of the luminescent system found in all luminescent cypridinid species worldwide (light production mechanisms and antipredatory displays), and 3) the features unique to the signaling clade (courtship displays) found only in the Caribbean. For a general overview of this luminescent system see Morin (1986) and Morin & Cohen (1991, 2010). Taxonomic descriptions of signaling species and genera can be found in Cohen & Morin (1986, 1989, 1993, 2010), Morin & Cohen (1988), Torres & Cohen (2005), and Torres & Morin (2007).

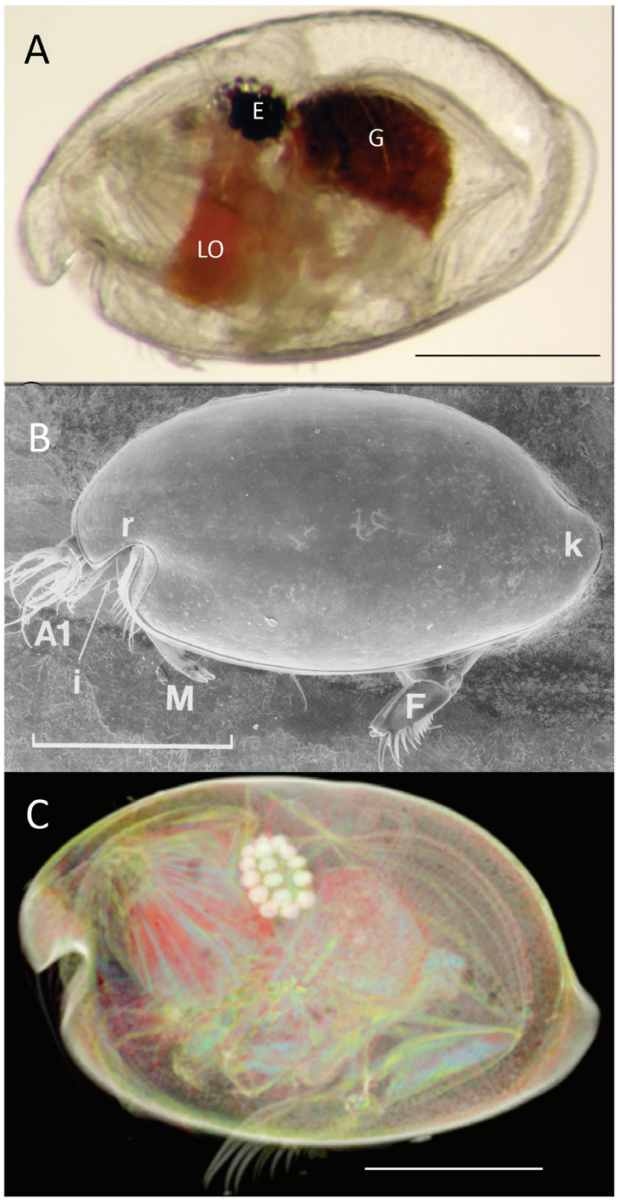

Figure 4. General morphology of representatives of the three main Caribbean clades of courtship-signaling cypridinid luminescent ostracods, shown using three micrographic technologies; scale bar, 0.5mm. Light microscope image of a live H-Group (undescribed genus) (**A**); scanning electron micrograph of a T–Group species (*Kornickeria louisi*; from Cohen & Morin, 1993: fig. 5A) (**B**); NanoCT scan of an F–Group species (*Photeros annecohenae*) (**C**). Abbreviations: A1, first antenna, E, left compound eye, F, furca, G, gut, i, incisur, k, keel, LO, light organ, M, mandible, r, rostrum. This figure is available in color at *Journal of Crustacean Biology* online. Used with permission.

Reproduction and life cycle of luminescent signaling ostracods

Most luminescent cypridinid ostracods are small and mostly benthic, the size of a sesame seeds or smaller, with adult males ranging 1.2–2.3 mm and females approximately 1.25 times larger (1.6–3.0 mm). They have large paired lateral eyes, with about 16 ommatidia, with those of the males being approximately 1.25 times larger than those of females. Except during their brief courtship display periods, individuals probably live within reefs, seagrass beds, or sediments near or below their courtship arenas, however, elsewhere in the world some cypridinids are pelagic or bentho-pelagic. During their courtship periods (see below) they become demersal with their signaling grounds just above the reef or seagrass bed.

Little is known of the feeding habits of most species, but at least a few are scavengers, coming to traps baited with fish tissue. None appear to be parasitic, despite many reports to the contrary.

Reproduction and development (Cohen, 1983; Gerrish & Morin, 2008).

Signaling ostracods appear to mate and reproduce throughout the year and show no seasonality. Fertilization is internal, with sperm being transferred by a spermatophore. The male eighth limb functions as a copulatory organ, which is enlarged and modified for grasping (Fig. 5A), whereas in females the eighth limb is reduced and includes a pair of knobs, probably grasped by males, and slits (Fig. 5B) (Cohen & Morin, 1993, 1997). The details of transfer of sperm and accessory materials are largely unknown. The spermatophore appears to act as a mating plug and probably blocks other male copulations (Fig. 5B).

All offspring in one brood are fertilized by the same male and, furthermore, females can store sperm and produce multiple broods from one insemination (i.e., one father) (Conley & Gerrish,

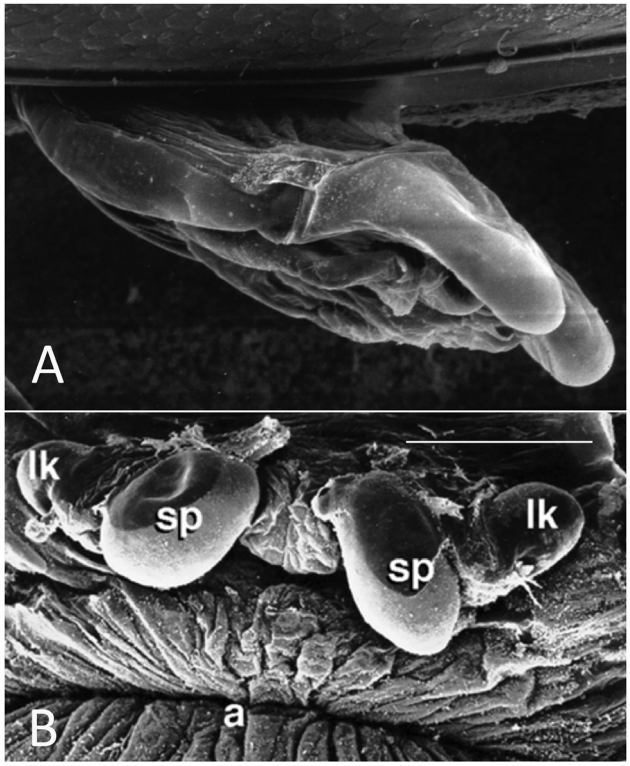

Figure 5. Copulatory (eighth) limbs. Male of undescribed H-Group species, right lateral view (**A**); female *Enewton harveyi*, ventral view (reprinted from Cohen & Morin, 2010: fig. 24A) (**B**). Abbreviations: a, anus; lk, lateral knob; sp, spermatophore. Used with permission.

2019). Females release eggs into the marsupium, the posterior-dorsal space between the enclosing carapace and the body, where the embryos are brooded (about 12 embryos) (Fig. 6). The mean sex ratio of each brood is equal between males and females. Adult females can produce several broods from one insemination.

Gestation lasts for a total of about 3.5 weeks. The fertilized embryos develop internally for about a week before the eggs are released into the marsupium, where they are kept for about another 2.5 weeks (Fig. 6). At this point the first instar offspring are released from the marsupium as crawl-away juveniles into the environment. New eggs develop in the female soon after larval release.

Juveniles molt five times over the course of about three months and the terminal (sixth) instar becomes the long-lived adult (Fig. 7), which can survive for up to six months. There is no pelagic larval stage, so initial dispersal is very restricted.

Diversity (Cohen & Morin, 1990, 2003; Torres & Gonzalez, 2007; Morin & Cohen, 2017). Displaying species appear to form a monophyletic clade with three major subclades (F, H, and T groups, and probably more (see above) (Fig. 3). Signaling species within this signaling clade are known only from across the Caribbean and they have been found nowhere else in the world's oceans. There are over 86 signaling species (and probably many more) in this geographically restricted signaling clade, all of which are initially identified from their displays and only about a quarter have been described (note one exception discussed above). More species are discovered whenever collections are made in a new island or regional location.

Endemism is high and separated islands or ecosystems have their own unique suites of species. Individual species show habitat and micro-habitat specificity.

Luminescence

Light production (Hastings & Morin, 1991; Morin, 2011; Wilson & Hastings, 2013). The luminescent reaction involves cypridinid luciferin (cypridinid LH_2 = a tripeptide of tryptophan, isoleucine, and arginine together forming a fused imidazopyrazine ring; see Fig. 16) + cypridinid luciferase (cypridinid Lase), which is a protein, + O_2 in seawater. The cypridinid luciferin and luciferase, along with mucus are ejected separately through specialized nozzles from separate large secretory cells situated in the upper lip, and mix in the sea water (Harvey, 1952; Huvard, 1993). As a result, light is extracellular, being produced external to the body of the ostracod. The light-emitting reaction follows simple first-order kinetics. The duration and intensity of luminescence vary depending on the relative proportions of LH_2 and Lase, Lase kinetics, and perhaps the relative amount of mucus, and the light is blue, centered around λ_{max} of 462 nm, but varies slightly among species (Hensley *et al.*, 2019).

Antipredatory displays (Morin & Cohen, 1991; Rivers & Morin, 2012). Predatory attacks (Fig. 8), which are rare, cause massive release of luminescence from either sex, all instars, and even late-stage embryos in the marsupium. The light produced during predation events releases about fifty times more luminescence (i.e., total number of photons) than a courtship display. Fish predators usually immediately regurgitate the ostracod unharmed after the ostracod is taken in and the light is released. The regurgitation behavior suggests a startle response, unpalatability, and/or aposematism in response to the light and ostracod. Ostracods appear to be unpalatable to the fish in all cases.

Courtship displays (*unique to the Caribbean signaling clade*) (Morin & Cohen, 1991, 2010; Rivers & Morin, 2008, 2009, 2013). Courtship displays are clearly a form of precopulatory mating selection between males and females. Sexually receptive (virgin) females respond to species specific male displays by swimming to intercept a signaling male in the water column, but without luminescing herself (Rivers & Morin, 2013). When small plankton nets are swept through an active display, usually only adult males and, at most, one receptive female are captured in this courtship arena. These arenas are equivalent to leks in the water column. Previously mated males remain sexually active and continue to signal, but mated females do not continue to be sexually receptive and do not take part in the courtship (Rivers & Morin, 2008). Instead, mated females become benthic and begin brooding embryos. As a result, because males remain signaling and mated females leave the courtship arena (lek), the operational sex ratio within the lek is highly skewed toward males even though the actual sex ratio in the population is near 1:1 (Rivers & Morin, 2008). This sex ratio bias leads to strong male-male competition (Rivers & Morin, 2009). Males produce various mating tactics, which vary from initiating a display, entraining on other displays, or sneaking without luminescing, yet there are no morphological differences among them, and even individual males will switch tactics during a single display event.

Courtship period (Gerrish *et al.*, 2009; Gerrish & Morin, 2016). Massive numbers of displays can occur in an area above a reef or seagrass bed from hundreds of signaling males in the display arena at night (Fig. 1). Displays begin either following sunset or near the setting of a waxing moon, whichever occurs first. Highest densities are observed when there is no moon present in the sky. Displays take place just at the end of twilight during the two weeks of the waning moon (i.e., the two weeks following the full moon), or later and later each night as the moon sets during the two weeks of the waxing moon (i.e., the two weeks after the new moon). Initiation of displays is quite precise, within minutes, and appears to track an isolume (particular ambient light intensity of the sky) as dusk or moonset occurs. This general light level centers around the start of astronomical twilight at dusk (40–60 min post-sunset) or during the waning phases of the moon cycle when

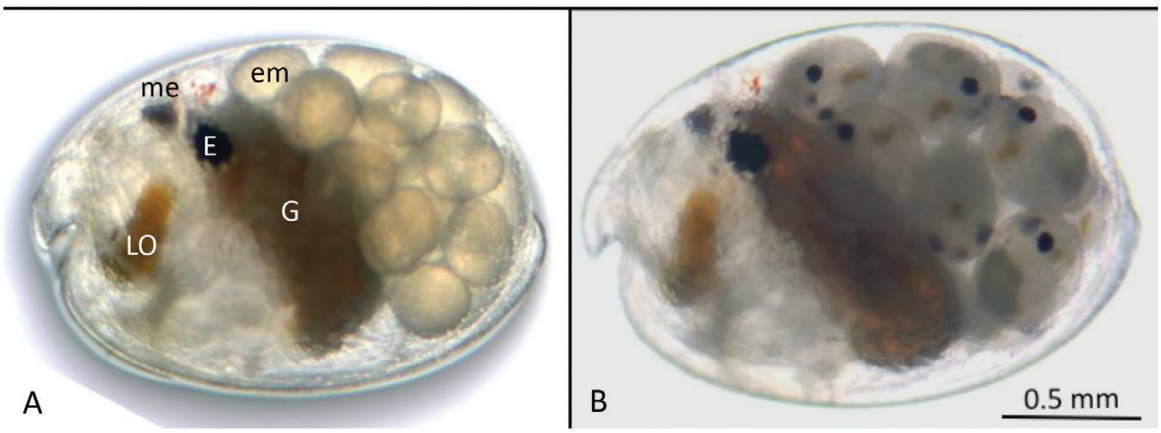

Figure 6. *Photeros annecohenae*, brooding adult females (modified from Gerrish & Morin, 2008: fig. 4b, e). Early brooding (**A**); late brooding, with compound eyes and light organs visible in embryos (**B**). Abbreviations: E, compound eye; em, embryo; G, gut; LO, light organ; me, medial eye. Used with permission.

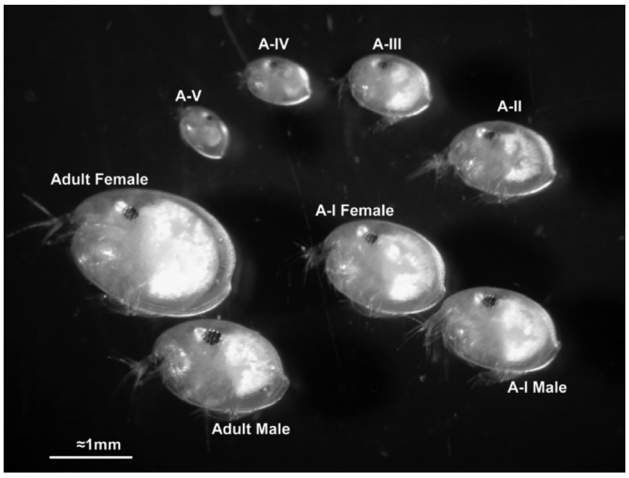

Figure 7. Five sequential juvenile instars (A–V to A–I`) and adult (sixth) stage of *Photeros annecohenae* (from Gerrish & Morin, 2008: fig. 3) showing size dimorphism of larger female and smaller male of both the fifth stage juvenile (A-1) and adult stages; scale bar = 1 mm. This figure is available in color at *Journal of Crustacean Biology* online. Used with permission.

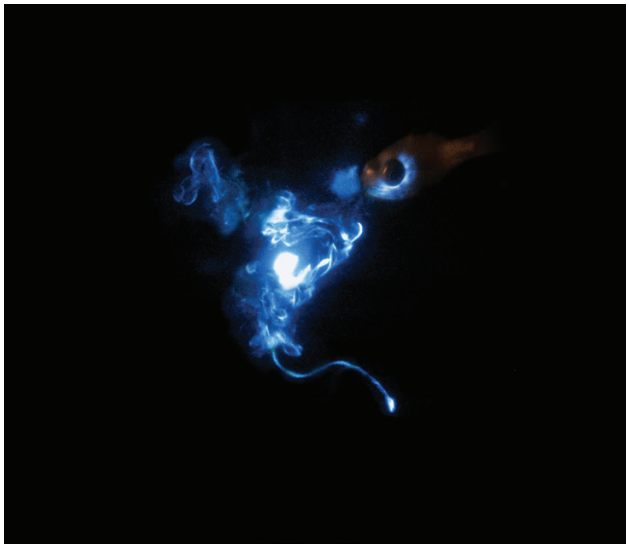

Figure 8. Antipredatory luminescent display ejected during a predation attempt of a dusky cardinalfish, *Phaeoptyx pigmenteria*, on the luminescent ostracod *Photeros annecohenae* (from Rivers & Morin, 2012: cover photo). The ingested ostracod was expectorated and is seen swimming away unharmed at the bottom of the luminescent cloud. Photo © Martin Dohrn, Ammonite Films, used with permission. This figure is available in color at *Journal of Crustacean Biology* online.

light levels are changing rapidly. By tracking a particular isolume, males of a particular species are consistently able to initiate displays from night at the same level of darkness whether it be the end of twilight during the waning phases of the moon or near moonset during the waxing phases of the moon. Furthermore, display initiation appears to be isolume consistent within species, but varies slightly among species. It can also vary somewhat within species with the amount of cloud cover, a few minutes earlier during heavy cloud cover and a bit later when it is clear.

Luminescent courtship display trains (Morin & Cohen, 1991, 2010; Rivers & Morin, 2008; Gerrish & Morin, 2016). Displays (Fig. 1) consist of a series of externally secreted light pulses, which together form a series of coded luminescent spots, usually with decreasing pulse intensity and duration, and decreasing interpulse intervals and interpulse distances from beginning to end. Displays are unique for each species in pulse number, train direction and duration, apparent speed, pulse durations, interpulse distances, and interpulse durations (Fig. 9). Trains are regularly repeated by males during the display period. Males swim rapidly (usually 8–11 cm s^{-1} = > 50 body lengths s^{-1}) in a tight helix.

Each display is usually composed of two phases: an initial alerting or species recognition phase and a final trill or tracking phase (Fig. 9). The initial (alerting/species recognition) phase usually consists of a few pulses that are brighter, of longer duration, and relatively widely spaced, while the secondary (trill/tracking) phase usually consists of many pulses that are dimmer, of shorter duration, and more regularly and closely spaced. The relative time allotted to the two phases often differs among species, with the initial phase dominating in some species, the trill phase dominating in others, and all situations in between being observed.

Luminescent Courtship Train Variations (Morin, 1986; Morin & Cohen 1991, 2010; Gerrish & Morin, 2016). Displays vary among species (Fig. 9), but little within any particular species. Train direction can be vertical up or down, oblique, or horizontal. Train lengths vary among species from only a few centimeters to many meters. Train durations vary from short (< 5 s) to long (nearly 1 min). Pulse numbers vary between one or two to hundreds per display. Displays can occur almost anywhere in the water column from near the substratum to high in the water column, even up to the surface in shallow water. Displays are habitat- and microhabitat-specific. The combination of all these characteristics allows for unique, quantifiable identification of each species display, a kind of display "fingerprint" (Fig. 9).

THE INDIAN OCEAN, OSTRACODS AND LUMINESCENCE

Shoals of pelagic luminescent ostracods in *Cypridina* H. Milne Edwards, 1840 are known to be common in the northwestern Indian Ocean, including the Arabian Sea, the Malabar Coast of India, and the Maldives (Kelly & Tett, 1978; Daniel & Jothinayagam, 1979; Herring, 1985), so it is not surprising that this region is where observations of luminescence emanating from ostracods were first reported by mariners. In the first published reports, Godeheu de Riville (1760, as translated in Godeheu de Riville, 1768) made the following observations between the Malabar Coast and the Maldives (ca. 8°47′N, 75°20′E) in 1754: "… [the sea] was covered over with small stars; every wave which broke about us dispersed a most vivid light, in complexion like that of a silver tissue electrified in the dark." He managed to secure some specimens and had remarkably detailed illustrations drawn (Fig. 10B), especially for the time. They were even better than some of the original descriptive illustrations produced by systematists nearly a century later (see below) (e.g., H. Milne Edwards, 1840). Furthermore, his are the first published drawings of any myodocopid ostracod. The illustrations are clearly of myodocopids, replete with brooding embryos, and likely a species of *Cypridina*, which is common there today.

GLOBAL OSTRACOD SYSTEMATICS AND TAXONOMY EXPLODES IN THE 19TH CENTURY

The first ostracods

Surprisingly, probably because of their generally small size, ostracods, whether myodocopids or podocopids, only became formally known to science during the latter half of the 18th century at about the same time as Godeheu de Riville's luminescence observations (Godeheu de Riville, 1760) and concurrent with development of binomial nomenclature by Linnaeus (1758). Ostracods, along with other crustaceans, were considered to be insects at the time of Linnaeus, but molecular phylogenetics have since clearly shown that Ostracoda is a monophyletic and ancient clade within

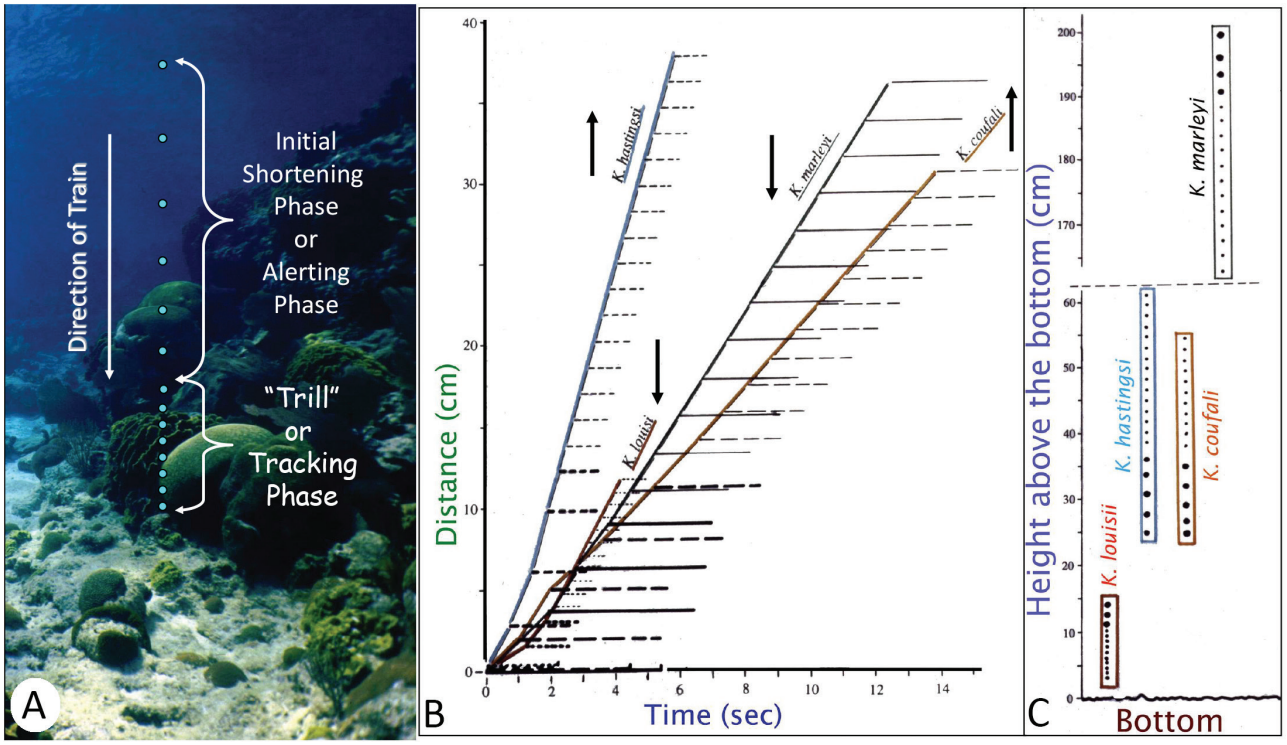

Figure 9. Courtship signal "fingerprint." Artist's depiction of the initial (= alerting) and trill (= tracking) phases of a typical slow downward luminescent courtship display (**A**). Time-distance relationships of luminescent courtship display trains for four species of *Kornickeria* from Jamaica (**B**); successive data points for each species represent distance traversed (cm) and time elapsed (s) since the prior pulse; each pair of horizontal points represents the start and end of a pulse and the horizontal line between represents the duration of a pulse; the slope of the line that connects the start of each pulse indicates the apparent (but not the actual) swimming speed; each arrow indicates the direction in which the display is produced in the field; pulse duration lines are offset from zero at the initiation pulse for clarity of comparison. Spatial relationships and direction of the displays shown in B relative to substratum (**C**). Data in B and C are derived from Cohen & Morin (1993). This figure is available in color at *Journal of Crustacean Biology* online.

Crustacea (Horne *et al.*, 2005; Regier *et al.*, 2010; Oakley *et al.*, 2012), and, ironically, that the Insecta are now merely a clade, albeit a huge one, within the Crustacea! The first known illustration of ostracods (a species of podocopid) was by Henry Baker (Baker, 1753) (Fig. 10A). While Linnaeus and others clearly mentioned ostracods (probably referring to freshwater podocopids) in their writings (see Skogsberg, 1920), none were detailed enough to identify accurately later. All the early descriptions were made using dried specimens, which often suffered severe distortion and loss of parts. The first to be described in some detail, by Otto F. Müller (1776, 1785) (Fig. 11A), were two podocopid ostracods: *Cypris*, a ubiquitous freshwater podocopid, and then *Cythere*, a marine podocopid. It should be noted than no podocopid or freshwater ostracod is known to be luminescent. The taxon Ostracoda was not established until later, by Pierre André Latreille (1802, 1806).

Cypridina

More ostracod species, mostly freshwater podocopids, were being described during the early 1800s, while knowledge of the marine myodocopids lagged behind. Henri Milne-Edwards (1840) (Fig. 11B) described the first myodocopid, *Cypridina reynaudi* (as *Reynaudi*) (Fig. 10C), which is perhaps the same species as that depicted by Godeheu de Riville (1768). It became the type, by monotypy, of *Cypridina*, but its description was incomplete. No museum specimens are known and the exact site of collection is uncertain, other than being listed from the Indian Ocean. Many generic and species descriptions were quite perfunctory prior to 1850, and sometimes beyond. William Baird (1850: 140) wrote "The descriptions found in the authors I have already quoted previous to him [O. F. Müller] were so superficial, that even when illustrated by figures, which were also generally very bad, there is no possibility of distinguishing what species they meant to describe. … In determining the species, therefore, we must consider the researches of the various authors previous to [O. F.] Müller as of little or no use whatever." Both the genus *Cypridina* and the type species need revising (see below).

Establishment of the myodocopid families

By the mid-1800s comparative differences among taxa were beginning to be recognized, more attention was being paid to details of morphology, descriptions of higher relationships were being formulated, and nomenclatural rules were being codified. Georg Ossian Sars (1866) (Fig. 11C), a giant among systematists of the time, established the subclass Myodocopa and order Myodocopida, which included Cypridinidae (Supplementary material Table S1), described many new genera and species, and set the standard for future ostracod taxonomy. The family Cypridinidae was established by Baird (1850), although at that time it included species now assigned to other families. It was not until nearly a half century later that G. Stewardson Brady & Alfred M. Norman proposed two quite distinctive families, Rutidermatidae and Sarsiellidae, separate from Cypridinidae (Brady & Norman, 1896) (Supplementary material Table S1). Gustav W. Müller (1906a), another significant contributor to ostracod studies (Fig. 11D), further separated the families Cylindroleberidae and Philomedidae from Cypridinidae. By early 20th century, therefore, the higher levels of myodocopid ostracod

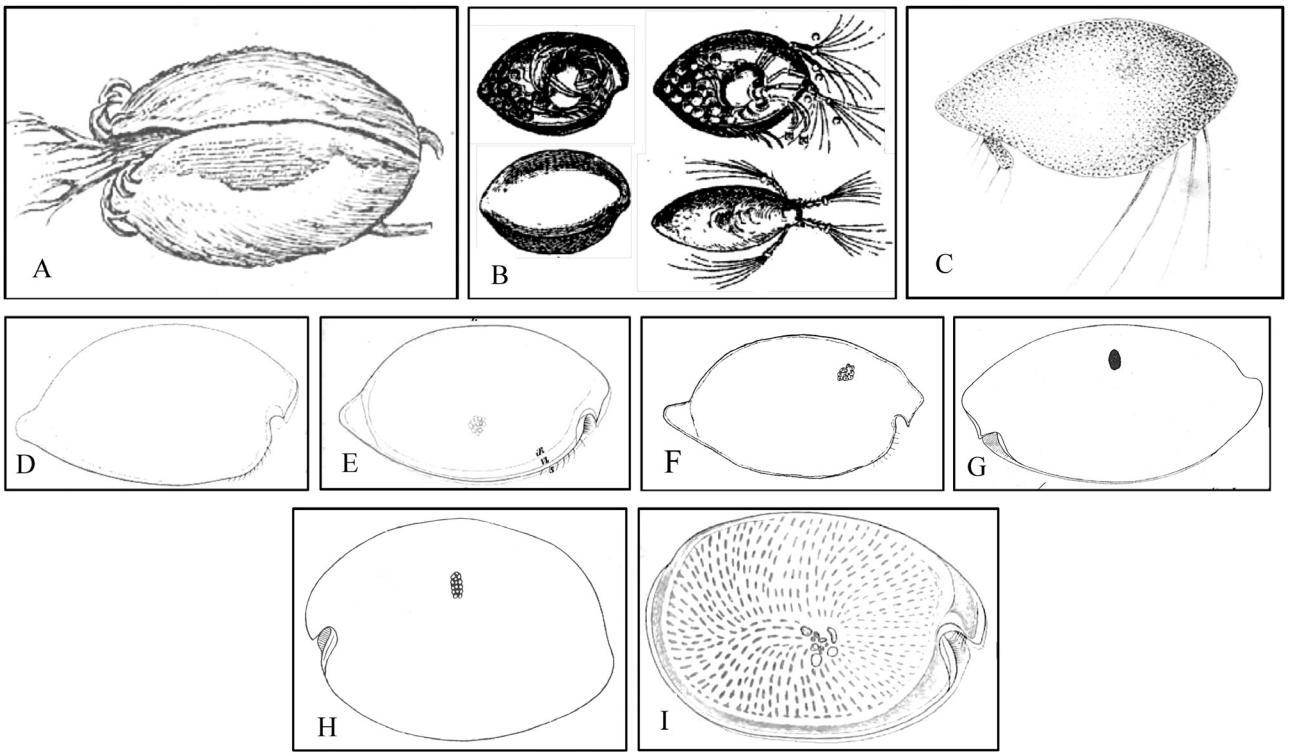

Figure 10. Comparative drawings of ostracods from the older literature. First known illustration of an ostracod in the published literature, a podocopid, by Baker (1753) (**A**). First known drawing of a myodocopid ostracod, a cypridinid, probably *Cypridina* sp., from observations of living specimens collected off the Malabar Coast, Indian Ocean, in 1754 by Godeheu de Riville (1760: figs. 2–4) (**B**). *Cypridina reynaudi*, type species of *Cypridina*, from H. Milne Edwards (1840: pl. 136, fig. 5) (**C**). Possible candidate species to be designated as *C. reynaudi* following redescription: *C. dentata* Müller, 1906b, from Müller (1906b: pl. 2, fig. 17) (**D**); *C. chierchiae* Müller, 1890, from Müller (1890: pl. 2, fig. 1) (**E**); *C. inermis* Müller 1906b, from Poulsen (1962: fig. 122) (**F**); and *C. serrata* Müller, 1906b, from Skogsberg (1920: fig. LVII-1) (**G**); for discussion of possible candidate species see Skogsberg (1920: 314–317, 330). *Vargula norvegica*, type species of *Vargula*, from Skogsberg (1920, fig. 38–5) (**H**). *Vargula hilgendorfii* = *umi hotaru*, from Müller (1890: pl. 25, fig. 9) (**I**). Permissions: A–E, G–I: public domain; F, Carlsberg Foundation.

systematics, including the families (Supplementary material Table S1), were firmly established (see World Ostracoda Database, World Register of Marine Species (WoRMS); http://www.marinespecies.org/ostracoda/). Of the five extant families currently constituting the order Myodocopida, only Cypridinidae (Table 1) are known to include luminescent species.

Splitting Cypridina *into five subgenera and subsequently raising them to genera*

The systematics of Cypridinidae changed rapidly between 1890 and 1920. Much of this expansion was triggered by the prodigious G.W. Müller (1890, 1906a, b, 1912, among other references) (Fig.11D), who described dozens of new myodocopid species and genera (Fig. 12). The number of species in the family swelled from 17 species in three genera to 42 species in seven genera (15 in *Cypridina* alone) by 1912. The evolution revolution was sweeping systematics around this time and, usually based on comparative morphology, relationships within and between families were being examined in light of the new theory. Evolutionary relationships were becoming clarified, new genera were being established, and old species were given new generic designations based on sound morphological grounds, which was typical of many groups, especially during this period. Tage Skogsberg (1920: 190) (Fig.11E) observed of *Cypridina* "The incompleteness and incorrectness that are characteristic of the descriptions of the majority of the species belonging to this genus result in our being able at present to submit these forms to only a comparatively superficial comparative investigation. But even a rather superficial study of them is, however, sufficient to show us that this genus comprises rather heterogeneous elements. It seems to have been a sort of lumber-room in which were thrown together all forms that it was impossible to arrange under any of the [other] genera…. G.W. Müller himself has pointed out the unnatural character of this genus and the urgency of splitting it up into smaller systematic units." Skogsberg (1920) proceeded to tackle this problem by carefully describing five new subgenera within *Cypridina*: the nomintypical subgenus *Cypridina*, along with *Doloria* Skogsberg, 1920, *Macrocyprdina* Skogsberg, 1920, *Siphonostra* Skogsberg, 1920, and *Vargula* Skogsberg, 1920, (along with many new species). *Cypridina* thereby became a well-defined subgenus, but *Vargula* became the new "lumber room," with diagnostic characters being rather disparate and ill-defined. By the time of the monumental monograph by Erik M Poulsen (1962), the number of cypridinid species had exploded to nearly a 100 and descriptions were being based on more and more detailed evidence. Poulsen (Fig.11F) took a bold step and raised Skogsberg's (1920) subgenera to generic rank.

Pyrocypris *synonymized with* Cypridina

G.W. Müller (1890) described a new genus, *Pyrocypris*, containing 13 species, all apparently luminescent. Subsequently, because his original descriptions of *Pyrocypris* species were rather poor and relied on the questionable character of shell pigmentation, and also because of the vagueness of H. Milne Edwards' descriptions of *Cypridina reynaudi*, the imprecise distributional data he provided, and the loss of specimens, determining nomenclatural priority and confirming synonymies between the various species of *Cypridina* and *Pyrocypris* proved very difficult (see lengthy discussion in Skogsberg (1920: 314–317)). Skogsberg (1920) was able

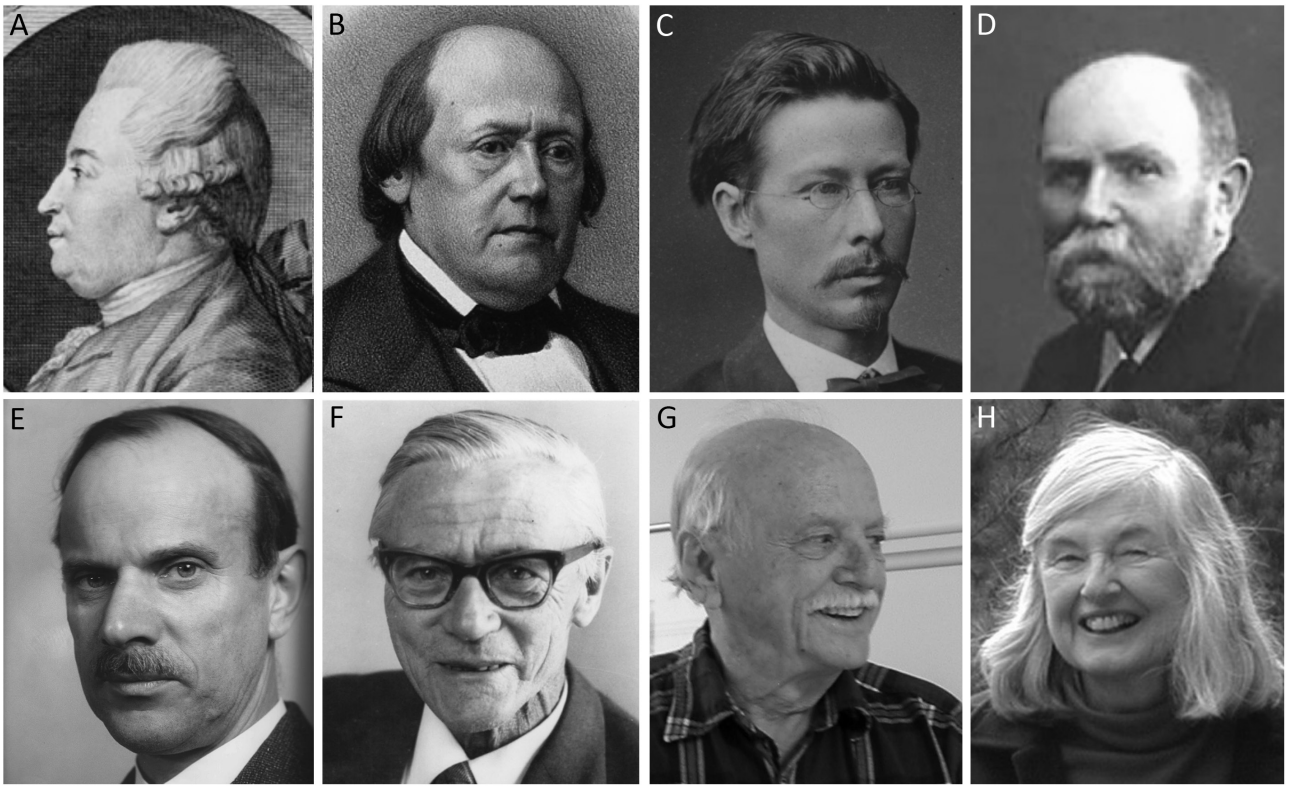

Figure 11. Portraits of pivotal and influential myodocopid ostracod systematists of the 18th through 20th centuries. Otto F. Müller (1730–1784) (**A**); Henri Milne Edwards (1800–1885) (**B**); Georg O. Sars (1837–1927) (**C**); Gustav W. Müller (1857–1940) (**D**); Tage Skogsberg (1887–1951) (**E**); Erick M. Poulsen (1900–1985) (**F**); Louis S. Kornicker (1919–2018) (**G**); Anne C. Cohen (1935–) (**H**). A–D from Wikipedia (public domain); E, with permission from the Harold A. Miller Library and Hopkins Marine Station, Stanford University; F, with permission from the *Journal of Crustacean Biology*; G, with permission from Anne Cohen; H. photo by JGM.

to synonymize *Pyrocypris* with the subgenus *Cypridina* by using the available descriptions and museum specimens, albeit in very damaged condition. Based on priority, *Cypridina* took precedence as the subgeneric name; however, because of incomplete descriptions Skogsberg equivocated and was not able to decide which of the various *Pyrocypris* species described by G.W. Müller corresponded to *Cypridina reynaudi*. Candidates (Fig. 10D–G) include *Cypridina chierchiae* (G.W. Müller, 1890), *C. inermis* (G.W. Müller, 1906), *C. dentata* (G.W. Müller, 1906) and *C. serrata* (G.W. Müller, 1906) as the type. With *Pyrocypris* made synonymous with *Cypridina*, the subgenus became rather homogeneous, especially compared to *Vargula*.

While many species were assigned to the original genus *Cypridina*, many were later reassigned to other genera or even families, especially after clarifications by Skogsberg (1920) (see also World Ostracoda Database; http://www.marinespecies.org/ostracoda/). Of particular note is *Cypridina norvegica* Baird, 1860 (Fig. 10H), a deeper-water luminescent species from near-Arctic European waters, which was described by Baird (1860) and which Skogsberg (1920) designated as the type for the (sub)genus *Vargula*. Another species was the now famous luminescent *Cypridina hilgendorfii* G.W. Müller, 1890 (Fig. 10I) from Japan, which was later transferred to *Vargula* by Poulsen (1962) (see below).

But even with the re-assortment of many species, from the humble beginnings of *Cypridina reynaudi* nearly 300 years ago, species now known to belong to the Cypridinidae have burgeoned to +250 (Fig.12, Table 1) and many more are known, but undescribed.

Improvements of descriptions and illustrations over time

Descriptions and illustrations of ostracods, as with many other taxa, have changed with time from rather crude sketches of only the prominent body parts such as mandibles and the fourth limbs (see Fig. 13A–D, E–H, respectively) to include all eight pairs of limbs and other structures in ever more informative detail as in the upper lip and copulatory limb (Fig. 14), especially as new technologies have become available. But even as late as 1920, after standard descriptive measures had become well established, Skogsberg (1920: 157) wrote "One must admit, unfortunately, that the method of description of species within this group is still at a rather low level." This situation has fortunately changed as descriptions have become much better, especially with the prodigious contributions of Louis S. Kornicker (Figs. 11G, 12), but still can be improved. Our examination of signaling species from the Caribbean, which we initially distinguished by differences in signal behavior, sometimes show only minor morphological differences between sister taxa. Anne C. Cohen (Fig. 11H) and I have shown that details of every available structure are often crucial in identifying species (Cohen & Morin 1993, 2010). Poulsen (1962: 9–10) provided yet another useful perspective to bear in mind when he wrote "It is quite true that, viewed with the eyes of our generation, a number of previous descriptions are 'poor,' in the sense that they are incomplete, not providing the information which we now find is needed, that they are inaccurate or even faulty. But it must be borne in mind that these descriptions, when given, served their purpose, rendering what at that time was judged necessary for the study of the species. – It is not ours to scorn at the work of the preceding generations – our limited comprehension of the background for it is prohibitive – ours is only to correct it where necessary just as the scientists of a future generation are expected to criticize what we may feel we have achieved. … They are all stones for the building of our knowledge, to be rearranged or even discarded as science advances."

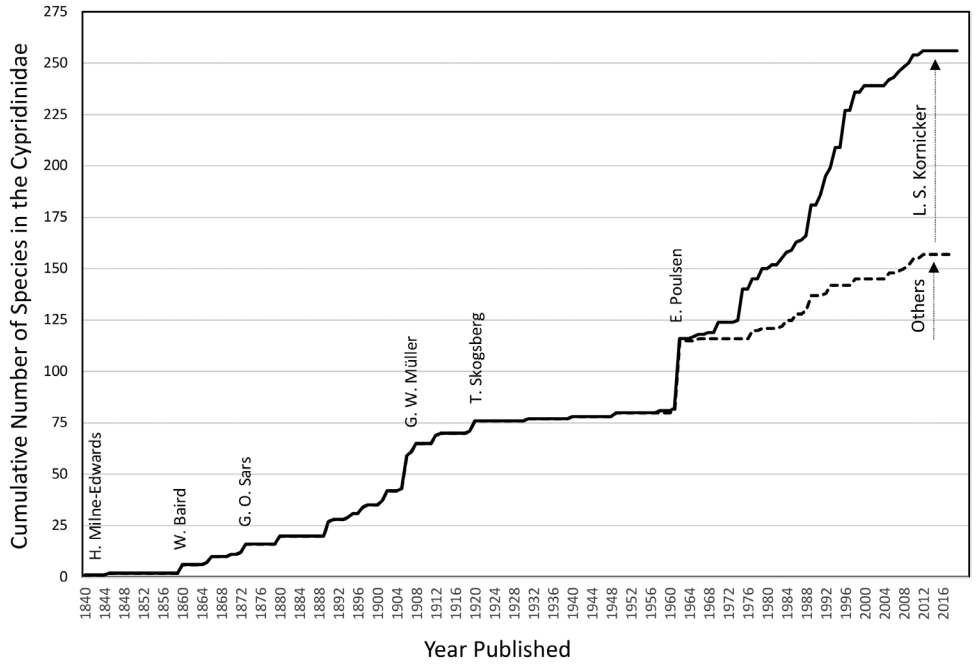

Figure 12. Cumulative number of species described in Cypridinidae from 1840 to the present; dashed line indicates species added by all contributors except L.S. Kornicker from 1958 through 2016; solid line indicates species added by all contributors including Kornicker. Especially large contributions to the species inventory were made by G.W. Müller, T. Skogsberg, E.M. Poulsen, and L.S. Kornicker. Note the effect of the depression and the World War II between 1920 and about 1960.

UMI-HOTARU (Vargula hilgendorfii) FROM JAPAN, E. NEWTON HARVEY AND THE PRINCETON GROUP

The most iconic of all luminescent ostracods is *Vargula* (formerly *Cypridina*) *hilgendorfii* from shallow waters of central Japan. It is locally known as *umi-hotaru* (海蛍), which translates to "sea-firefly." This ostracod was originally described as *Cypridina hilgendorfii* by G.W. Müller (1890) from material collected by Franz M. Hilgendorf while he was teaching in Japan and deposited by Hilgendorf in the Museum für Naturkunde, Berlin in 1876. The species was well known to the Japanese because of its luminescence and the fact that specimens could be readily caught in substantial numbers in shallow water on fish carcasses placed on the sea floor. To avoid confusion, I use the term *umi-hotaru* to refer specifically to *Vargula hilgendorfii* and no other luminescent ostracod. H. Watanabe (1897) indicated that light production in these tiny *umi-hotaru* occurs via a simple reaction involving only two substances that give off light when mixed in the presence of water, and that the light organ, located on the upper lip (labrum), contains two types of secretory cells, with each cell type presumably contributing one of the reactants. G.W. Müller (1890) also noted two secretory types cells in his initial descriptions of preserved specimens of *C. hilgendorfii*, and these observations have been consistently confirmed since then by various methods (Harvey, 1952; Huvard, 1993; Abe *et al.*, 2000).

It was E. Newton Harvey (Fig. 15A), however, who made *umi-hotaru* famous. After completing his Ph.D. in two years at Columbia University, New York under Thomas Hunt Morgan. His thesis, "The permeability of cells," was on a topic which he continued to study throughout his life. Harvey took a position as an Instructor at Princeton University in 1913 at the age of 23 (Johnson, 1967). He spent the next 43 years at Princeton, but spent most summers at the Marine Biological Laboratory at Woods Hole, MA studying the chemical mechanisms of bioluminescence among organisms. He also traveled widely pursuing his research questions on bioluminescence. He was very much a comparative physiologist and biochemist and was fascinated by the differences between the various organisms he studied. Bioluminescence is an excellent, non-invasive indicator of a chemical reaction. He began his monumental work on bioluminescence studying fireflies and bacteria, but that changed abruptly when he traveled to Japan on his honeymoon in 1916. There he visited the Misaki Marine Laboratory near Tokyo and was introduced to *umi-hotaru* (*C. hilgendorfii*) (Johnson, 1967; Anctil, 2018). This tiny organism produces copious bluish luminescence when disturbed, and it was known in Japan that if they were quickly dried in the sun and kept desiccated, their luminescence would return simply by adding water. If the dried ostracods were kept free of moisture with a desiccant, the luminescence would be preserved for indefinite periods (we now know that it is decades!). As a biochemist interested in the mechanisms of light production, Harvey was constantly seeking readily available, simple systems. Large numbers of individuals must be obtained for quantitative work on reaction mechanisms because purification always resulted in significant losses, thus yielding only minute quantities of the desired products, which themselves were often quite unstable and labile. Here, with *umi-hotaru*, was an abundant source of a simple light-emitting system that was stable prior to manipulation, and Harvey quickly capitalized on it. The simplicity, just two chemical compounds, and stability, especially compared to fireflies and bacteria, made the organism very attractive.

While in Tokyo in May, 1916, Harvey wrote an article about his comparative work with fireflies and bacteria (as well as other systems), but not his ostracod research in Japan, which had only just started, in which he stated "In a general way, … we may say that the problem of bioluminescence has been solved at least in its broad aspects. There still remain many details to be filled in, details which will take some time to complete. The exact chemical nature of luciferin is unknown, but the method of attack of the problem has been out-lined and all that is necessary is a sufficient quantity of the luminescent material for the determination of its chemical nature.… Luciferase, on the other hand, has all the properties of an enzyme, … an oxidizing enzyme which will

Table 1. Genera of Cypridinidae. Author and year of description, along with number of species in genus (number of described species, total number, number luminescent and which produce courtship signals, both described and undescribed); *, genera with species that produce courtship signals; **, 10 species currently described as *Vargula* that belong to one of undescribed genera in C, H, or Z groups.

Genus	Year described	Author	No. described species	No. described courtship signalers	No. undescribed courtship signalers	Total no. courtship signalers	TOTAL (described + undescribed)
Cypridina	1840	Milne-Edwards	46				46
Heterodesmus	1866	Brady	3				3
Eumonopia	1891	Claus	1				1
Gigantocypris	1895	Müller	6				6
Codononcera	1902	Brady	13				13
Cypridinodes	1902	Brady	18				18
Doloria	1920	Skogsberg	9				9
Macrocypridina	1920	Skogsberg	2				2
Siphonostra	1920	Skogsberg	2				2
*Vargula**	1920	Skogsberg	45	–10**			35
Azygocypridina	1950	Sylvester-Bradley	11				11
Hadacyprdina	1962	Poulsen	2				2
Paracypridina	1962	Poulsen	2				2
Bathyvargula	1962	Poulsen	2				2
Paradoloria	1962	Poulsen	13				13
Skogsbergia	1962	Poulsen	19				19
Melavargula	1962	Poulsen	2				2
Paravargula	1962	Poulsen	10				10
Pterocypridina	1962	Poulsen	10				10
Sheina	1966	Harding	1				1
Metavargula	1970	Kornicker	15				15
Isocypridina	1975	Kornicker	2				2
Rugosidoloria	1975	Kornicker	1				1
Rheina	1989	Kornicker	2				2
*Kornickeria**	1993	Cohen & Morin	5	5	20	25	25
Pseudodoloria	1994	Kornicker	1				1
Lowrya	1998	Parker	2				2
Jimmorinia	2000	Cohen & Kornicker	2				2
*Enewton**	2010	Cohen & Morin	1	1	0	1	1
*Photeros**	2010	Cohen & Morin	8	8	11	19	19
H/C/Z Groups *			0	10**	31	41	41
Totals - 30 genera			**256**	**24**	**62**	**86**	**318**

oxidize luciferin" (Harvey, 1916: 209). Little did he know then that the field of bioluminescence study had just scratched the surface. With his 1916 publication he laid out his research plan into the future, and, with the availability of *umi-hotaru*, he had an ideal system to carry it out. Through using *umi-hotaru*, he, his students, and colleagues produced dozens of insightful publications on the basic mechanisms of light production by organisms.

The first article by Harvey (1917) on the chemistry of light production by *umi-hotaru* came from that first visit to Japan. He opened the article with "By far the most valuable of any of these organisms [i.e., luminescent fireflies, bacteria, sea pens (pennatulacean cnidarians), and burrowing clams (pholads) with which Harvey and others had worked] … is the small crustacean, *Cypridina hilgendorfii*. For the size of animal, the light-giving substance is relatively enormous and its light-giving power incredibly great. Suffice it to say that one part of luminous gland substance in 1,600,000,000 parts of water will give visible light" (Harvey, 1917: 319). He returned to Japan the next year (1917) to further his ostracod research and, critically, managed to set in place a procedure to receive regular shipments of substantial volumes of dried *umi-hotaru* from Japan to Princeton. With this arrangement began four decades of research by Harvey and his graduate students, post-docs, and colleagues that deciphered many mysteries of how light is generated by these remarkable organisms. Harvey, the consummate comparative biologist, in parallel with his ostracod research, also continued to study bacteria, fireflies, sea pens, ctenophores, hydromedusans, fishes, shrimps, and annelids, and he wrote many reviews on luminescence, the two most notable of which were his compendious book *Bioluminescence* (Harvey, 1952), which reviewed the known literature on bioluminescent species, and *A History of luminescence* (Harvey, 1957), which followed the time-line of studies of luminescence.

THE MOLECULAR BIOLOGY OF CYPRIDINID LUMINESCENCE

It is important to remember that Harvey's endeavors started more than a 100 years ago. There were few tools available to unravel the mysteries of the mechanisms involved in biochemical reactions. Absent were techniques we view crucial today. There were no electrophoretic, chromatographic, or centrifugation methods used for isolating and purifying the compounds, no photomultipliers to quantify light measurements, no

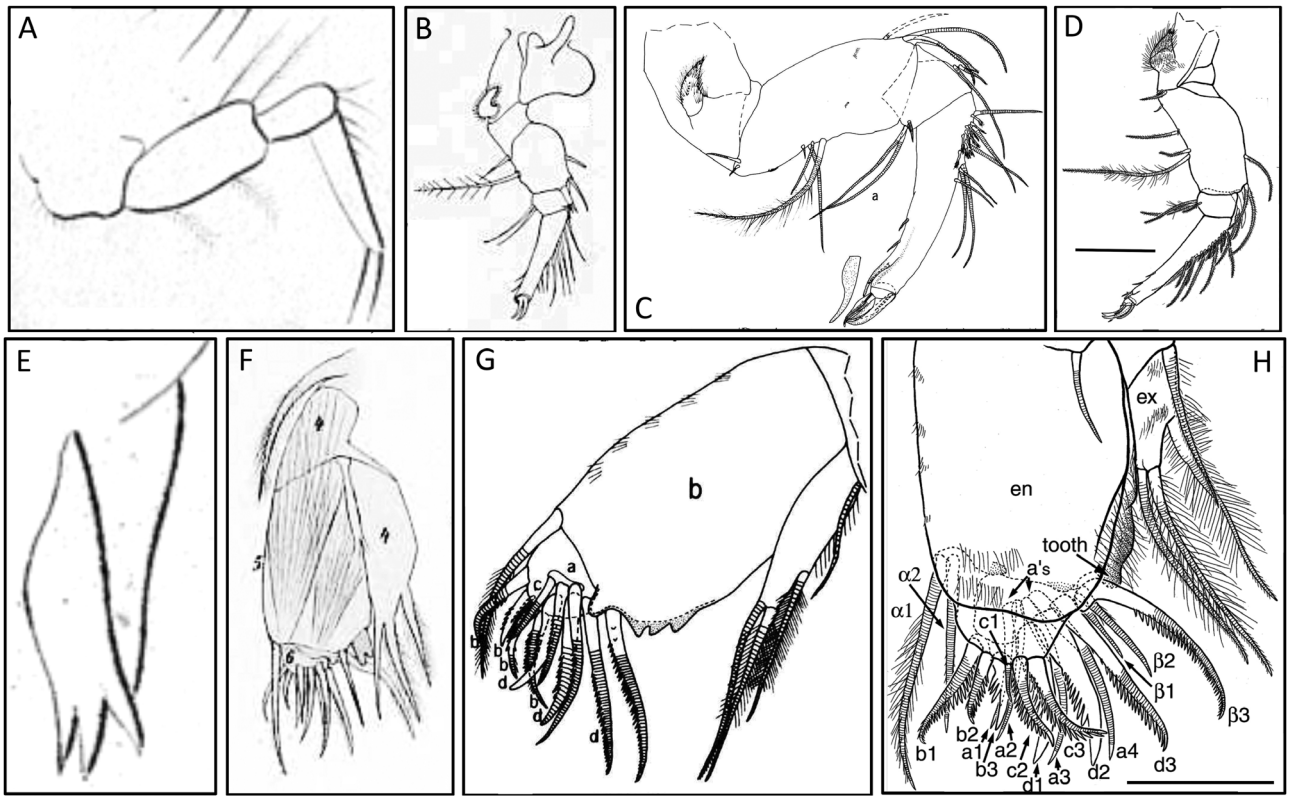

Figure 13. Changes through time in detail provided in illustrations, exemplified by mandibles (A–D) and fourth limbs (first maxillae) (E–H). *Cypridina reynaudi*, from H. Milne Edwards (1840: pl. 36, fig. 5d) (**A**); *Photeros parasitica*, from Wilson (1913: pl. 53–307) (**B**); *Kornickeria bullae*, from Kornicker (1984: fig. 10a) (**C**); *Photeros mcelroyi*, modified from Cohen & Morin (2010: fig. 13F) (**D**); *Cypridina reynaudi*, from H. Milne Edwards (1840: pl. 36, fig. 5e) (**E**); *Vargula hilgendorfii*, from Müller (1890: pl. 27, fig. 2) (**F**); *Vargula magna*, from Kornicker (1984: fig. 16b) (**G**); *Enewton harveyi* from Cohen & Morin (2010: fig. 15A) (**H**). Scale bars = 200 μm (D), 100 μm (H).

ultraviolet-visible spectrophotometry to measure the wavelengths of absorbed, reflected, or emitted light that reveal structure, and no x-ray crystallography to determine organic molecular structure. It was during the next four decades, from the 1920s to the 1950s that most of these tools became available, and the Princeton group was often at the forefront of adopting these methods (summarized in Harvey, 1952). Harvey's Princeton group became a powerhouse of the day. Along the way Harvey and colleagues used cutting-edge technology to answer critical questions and pave the way for ever newer technologies. They employed many revolutionary techniques, which may seem quaint by today's high-tech standards, that allowed steady progress, sometimes with dead-ends and backtracking, in their quest to understand the chemical basis for light emission from organisms. New discoveries continue today through the work of Harvey's academic descendants and others.

The critical first steps in working with *umi-hotaru* showed that the luminescent reaction was indeed quite simple: it was an oxidative reaction in an aqueous environment that involved only 1) an enzyme (cypridinid luciferase), which was heat-labile, 2) a substrate (cypridinid luciferin), which was heat-stable, and 3) oxygen. He could easily make hot water (= substrate luciferin) and cold water (= enzyme luciferase) extracts, which when mixed together produced light. Once this basic reaction was understood, his next critical questions focused on specificity and purity. Early on Harvey devoted a lot of his efforts to both these problems.

Specificity

Harvey performed many hot/cold cross-reactions experiments with other organisms, including many luminescent forms, and various animal derivatives (Harvey, 1952). The results showed that the reaction was highly specific to cypridinid ostracods and that neither the luciferase nor the luciferin would cross-react with other luminescent systems.

Purification

Harvey also continued his quest to gain more and more purified products, first focusing on the cypridinid luciferin and later the luciferase. Only upon having purified substrate and enzyme could their structure and properties be determined with precision. This problem occupied much of his later work and that of his students, including Rupert Anderson, Aurin Chase, Howard Mason, Fred Tsuji (Fig. 15B), and indirectly, Osamu Shimomura (Fig. 15C). The final structure of cypridinid luciferin was reported by Shimomura and associates (Kishi *et al.*, 1966a. b, c, d), and the purification, characterization, and cDNA cloning and sequencing of cypridinid luciferase was accomplished between the early 1950s and 1989 by a number of researchers (see Shimomura (2006), Wilson and Hastings (2013), Anctil (2018) and Shimomura *et al.* (2018) for general accounts of ostracod and other luminescent systems and methods).

Cypridinid luciferin

A key breakthrough in purifying cypridinid luciferin came when Anderson (1935) devised a method that increased by 2,000× the light per weight of luciferin. It was a two-step process that first made this unstable molecule fat-soluble so water-soluble impurities could be removed, and then converted it to a water-soluble molecule so fat-soluble impurities could be removed. Later chromatographic and spectrophotometric methods by Chase, Mason, Tsuji, and others during the 1940s and 1950s further improved the

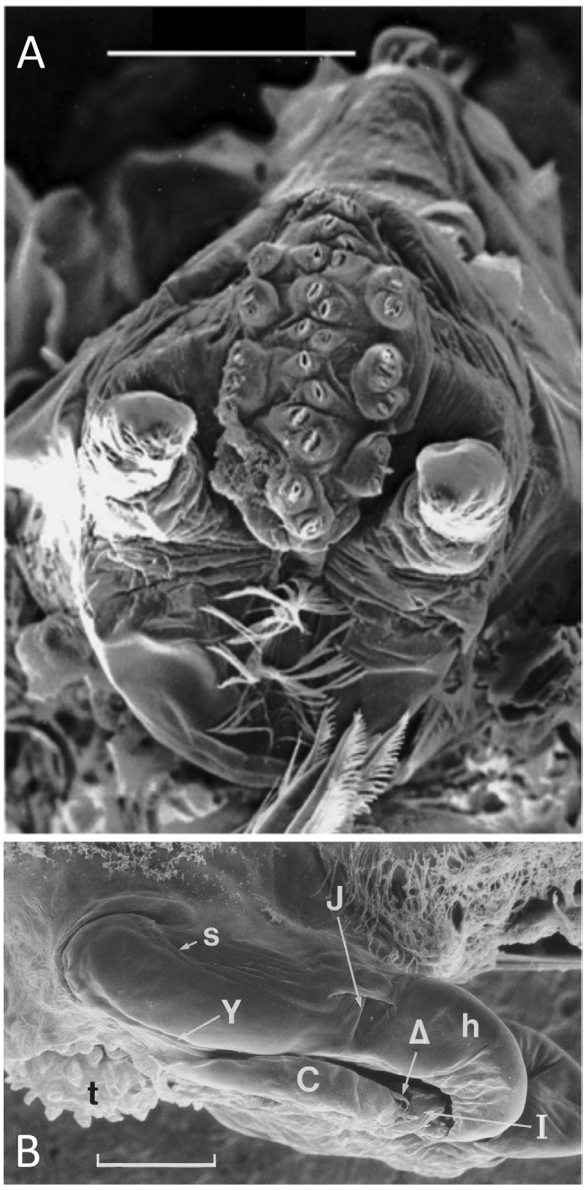

Figure 14. Upper lip and copulatory limb of luminescent signaling cypridinid ostracod species. *Photeros jamescasei*, ventral view, from Cohen & Morin (2010: fig. 8E) (**A**); *Kornickeria louisi*, right lateral view, from Cohen & Morin (1993: fig. 21A) (**B**). Abbreviations: C, central lobe, h, hood, I, inner lobe, J, joint between Y-sclerite and thumb sclerite, s, strut, Y, Y-sclerite, Δ, diagnostic generic character. Scale bar = 50 μm. Used with permission.

purity and allowed partial determination of its structure as a polypeptide that contained nitrogen and a reactive ring. But it was not yet pure enough to form crystals, which would allow a definitive structural determination via UV-visible absorption spectroscopy. The breakthrough came in 1956 when Shimomura, working in the laboratory of Yoshimasa Hirata at Nagoya University, Japan, produced the first crystals of pure cypridinid luciferin (Shimomura *et al.*, 1957). Determination and synthesis by the Nagoya Group in Japan (O. Shimomura, T. Kishi, T. Goto, and T. Hirata) followed during the next ten years. They showed that cypridinid luciferin is a tripeptide with a fused imidazopyrazine ring composed of tryptophan, isoleucine, and arginine (Kishi *et al.*, 1966a) (Fig. 16). Kishi, Goto, and other members of the Nagoya Group synthesized cypridinid luciferin and refined the structure (Kishi *et al.*, 1966b, c, d). While Shimomura never worked directly with Harvey (Harvey died suddenly in 1959), he joined the laboratory of Frank Johnson (who was Harvey's heir apparent) for three years at Princeton in 1960–1963 on a Fulbright Fellowship, where his focus shifted to the aequorin/GFP system in cnidarians and ultimately earning him the Nobel Prize in Physiology or Medicine in 2008. After a brief stint on the faculty at Nagoya University back in Japan, Shimomura permanently moved to Princeton in 1965, where, much like Harvey, he carried on his broad ranging biochemical studies of bioluminescent organisms, especially the aequorin/GFP system found in cnidarians (Shimomura, 2006; Shimomura *et al.*, 2018). He moved to the Marine Biological Laboratory at Woods Hole in 1981 until 2017 when he moved back to Nagasaki, Japan. Shimomura died on 19 October 2018 at the age of 90.

Cypridinid luciferase

Studies of the properties of cypridinid luciferase continued along with those of cypridinid luciferin but progressed more slowly. In a major step, the purification and many of the significant properties of the enzyme were published in back to back publications by two independent research groups (Shimomura *et al.*, 1961; Tsuji & Sowinski, 1961). The cDNA was subsequently cloned and sequenced by Eric Thompson, Shigekazu Nagata, and Fred Tsuji (Thompson *et al.*, 1989) working jointly in Japan and the United States. Cypridinid luciferase is a monooxygenase formed by a single acidic polypeptide chain of 555 amino acids, with a molecular weight of about 62kDa (see also Tsuji *et al.*, 1974). See Shimomura (2006) for a more complete documentation of the details and references.

The luminescent reaction

The cypridinid luminescent reaction involves only the substrate, the enzyme, and O_2 in a three-step process whereby 1) the luciferin, bound to the luciferase, is oxygenated to form a cyclic peroxide (dioxetanone), which 2) decomposes to oxyluciferin in an excited state (indicated by the asterisk in the reaction below) and releases CO_2, and 3) the excited electron in the oxyluciferin then emits a photon of blue light when the electron falls back to its ground state:

cypridinid luciferase + cypridinid luciferin + O_2 $\xrightarrow{1}$
cypridinid luciferase-cypridinid luciferin-dioxetanone intermediate $\xrightarrow{2}$
cypridinid luciferase-cypridinid oxyluciferin* + CO_2 $\xrightarrow{3}$
cypridinid luciferase + cypridinid oxyluciferin + $h\upsilon$ (λ_{max} ~ 462nm)

There are two other noteworthy light-emitting relationships involving ostracods. The first system involves marine fishes. While the cypridinid luciferin-luciferase system appears to be unique to cypridinid ostracods, at least three groups of fishes, species of *Porichthys* (the midshipmen, family Batrachoididae) along the west coast of North America and in the Caribbean, and *Apogon* (cardinalfishes, family Apogonidae) and *Parapriacanthus* (sweepers, family Pempheridae) in the Western Pacific utilize cypridinid luciferin to emit their own light, but in conjunction with different oxygenases as the enzyme (Haneda & Johnson, 1958; Haneda *et al.*, 1966; Tsuji *et al.*, 1972). These fishes most likely co-opt the substrate from their ostracod prey by dietary transfer. The details of the respective enzymes in comparison to those of cypridinids are unknown and should be explored.

A second system involves another group of myodocopid ostracods, the pelagic halocyprids, also emits luminescence (Angel, 1968), but is intracellular rather than extracellular, is of a different color (green, rather than blue), and light emission occurs by a very different biochemical mechanism involving different enzymes and substrates. This system represents an independent evolution of light emission (Campbell & Herring, 1990; Oakley, 2005). These halocyprids utilize the phyletically widespread luciferin molecule coelenterazine, which, like cypridinid luciferin, also has

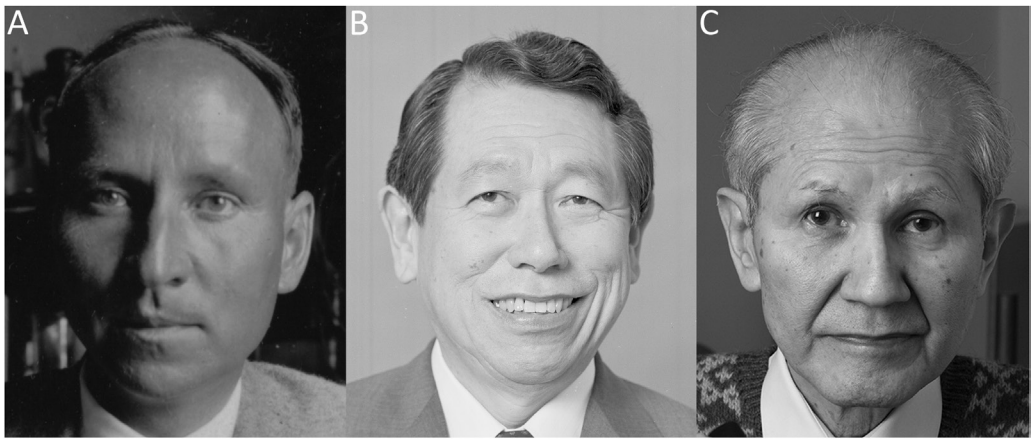

Figure 15. Portraits of three of the most notable contributors to the elucidation of the chemistry of the cypridinid luminescent system and the structures of cypridinid luciferin and cypridinid luciferase. E. Newton Harvey (1887–1959) (**A**); Frederick I. Tsuji (1923–2016) (**B**); Osamu Shimomura (1928–2018) (**C**). A used with permission from the Marine Biological Laboratory, Woods Hole, MA; B with permission from the Special Collections & Archives, University of California, San Diego; C. with permission from the Marine Biological Laboratory, Woods Hole and Tom Kleindinst.

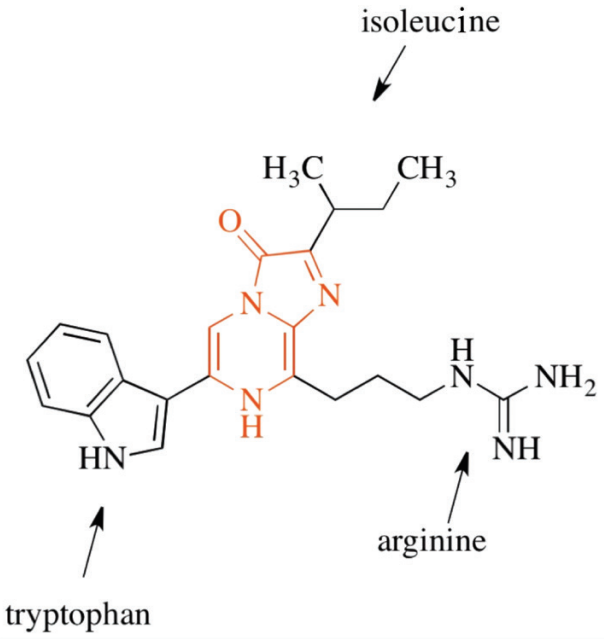

Figure 16. Structure of cypridinid luciferin, which consists of a fused imidazopyrazine core assembled from tryptophan, isoleucine, and arginine.

a fused imidazopyrazine ring core, but is composed of three distinctly different amino acids (two tyrosines and phenylalanine) and clearly has a different evolutionary origin from cypridinid luciferin, which, as noted above, is composed of tryptophan, isoleucine, and arginine.

WHEN SYSTEMATICS AND BIOCHEMISTRY APPARENTLY CONFLICT

The systematics of cypridinid ostracods has changed radically since H. Milne Edwards (1840) description of *Cypridina reynaudi*, the type species for the genus (Fig. 10). A half century later G.W. Müller (1890) described *umi-hotaru* as *C. hilgendorfii*. Prior to the work of Skogsberg (1920), the genus *Cypridina* was ill-defined and included a number of rather disparate species. Skogsberg carefully reassigned the species into five more narrowly defined subgenera but left one, *Vargula*, as the least precisely diagnosed; it became the new "lumber room" (see above). *Umi-hotaru*, which clearly did not belong in the same subgenus as *Cypridina reynaudi*, was placed in this new subgenus as *Cypridina* (*Vargula*) *hilgendorfii*. Poulsen (1962), based again on sound nomenclatural rules, raised the five subgenera to the genus level, whereupon the scientific name of *umi-hotaru* became *Vargula hilgendorfii*.

This change has caused consternation among biochemists ever since. As is often the case, appropriate changes in one area of science are often not quickly adopted in more distant branches. *Vargula* as the correct genus designation for *umi-hotaru* did not begin to penetrate the biochemical literature for 25 years (see Thompson *et al.*, 1987), and even then, it was sporadic. The problem continues to persist even today over 50 years later (see Shimomura, 2006)!

Of course, along with our incremental biochemical understanding of luminescence in cypridinids, the systematics of luminescent species has also evolved immensely since 1962. Of the five genera elevated by Poulsen (1962), *Cypridina* contains about 46 species, all of which are luminescent; *Vargula* currently has 45 species, probably most of which are luminescent, and there are 13 non-luminescent species distributed among *Doloria*, *Siphonostra*, and *Macrocyprdina*. The entire family now contains over 250 described species in 30 genera (Table 1), and their evolutionary relationships are becoming established, especially with the application of both molecular and morphological data (Fig. 3). Roughly half the species are luminescent, and the Caribbean luminescent signaling clade, which appears to be monophyletic and does not truly belong in either *Cypridina* or *Vargula* (Cohen & Morin, 1990, 2003; Torres & Gonzalez, 2007), contains about half of the known luminescent species (most of which are known but yet undescribed).

Some of today's most prominent biochemists still refuse to adopt their nomenclature to current taxonomy. Shimomura, who next to Harvey did more than anyone to advance our understanding of the mechanisms of light production in cypridinids, wrote in his book *Bioluminescence: chemical principles and methods* (2006: 50) "In this book, the classification of ostracods by Müller (published between 1890 and 1912) is used; the genus names by the Poulsen classification are cited in parentheses only when appropriate. The author protests against irresponsible shuffling of scientific names, and hopes for the revival of the Müller classification. In addition, the author believes that '*Cypridina* luciferin' is the proper name of this substance that is not necessarily bound to the genus name." It is unfortunate that Shimomura seemed not to understand that the changes in scientific names is not "an irresponsible shuffling," but rather a serious attempt to provide accurate information about the correct

phylogenetic relationships, based on established criteria, among this remarkable group of ostracods. Furthermore, as pointed out above, even G.W. Müller recognized that *Cypridina* needed to be split up! There is no going back to Müller's classification any more than biochemists might revert to using phlogiston as a way to explain the mechanism of combustion.

Many existing species diagnoses nevertheless still require more thorough descriptions. Such descriptions will almost certainly mean additional name changes in the future as relationships become even better clarified, but those changes will almost certainly be minimal modifications of the general phylogenetic picture that is in favor today. *Umi-hotaru* is a very distinctive ostracod that is likely to be given yet another generic designation in the future. It certainly does not belong in *Cypridina*, but neither does it belong to *Vargula* (Cohen & Morin, 1990, 2003; Torres & Gonzalez, 2007). One easy way that I proposed (Morin, 2011) is for biochemists to label the luminescent molecules of cypridinid ostracods by giving them the prefix "cypridinid," the colloquial name for the family, rather than *Cypridina* or *Vargula*.

THE CARIBBEAN LUMINESCENT COURTSHIP CLADE

New technologies, just as in the past, have allowed us to explore in ever more detail the vast array of taxa that constitute the Ostracoda. Relationships are becoming clear. With improved techniques such as scanning electron, fluorescence, confocal, and other microscopy, non-invasive nano/microCT imaging (see Supplementary material Videos S2, S3), better collection and preservation, and genetic sequencing including transcriptomics, the opportunity to achieve exact taxon sorting is now virtually assured. With the discovery of a flock of nearly a hundred closely related species in the Caribbean that are distinct from other genera in Cypridinidae, the systematics of the family, and perhaps all ostracods, has entered a new era in which precise details matter. Many species in the signaling clade are very difficult to distinguish from one another on morphological grounds, but are relatively easy to separate based on their courtship behavior and distributions (Fig. 9). This clade would seem to offer an ideal testing ground for studying the limits of many of these new investigative techniques. One wonders how many more possible flocks of ostracod species, likely driven by sexual selection (Ellis & Oakley, 2016) on some specific set of traits similar to, but different from, luminescent signals, might be waiting to be discovered among ostracods in general. For example, chemical and mechano-sensory behaviors are virtually unstudied in ostracods (e.g., Parker, 1995).

Based on the necessary details required to describe signaling species, ostracod systematics is in need of refinement. For instance, none of the species in the signaling clade truly belong in *Vargula*, even though many are assigned there in the absence of current generic diagnoses and will have to be reassigned. Furthermore, prior to 1980 only four species of luminescent ostracods, all described in *Vargula*, were known from the Caribbean. These are currently designated as *Photeros parasitica* (Wilson, 1913) and *P. harveyi* (Kornicker & King, 1965) from Jamaica (see Cohen & Morin, 2010), *Kornickeria bullae* (Poulsen, 1962) from moderate depths (> 25 m) off the U.S. Virgin Islands (see Cohen & Morin, 1993), and *V. magna* Kornicker, 1984 from deep water (160–202 m) on the continental shelves of North Carolina and West Florida, which probably does not belong to the signaling clade.

Since the discovery in 1980 that cypridinid ostracods use luminescent signals for courtship, major progress has been achieved in determining the biology of their mating system and their systematics, and new taxonomic standards have been established. Signal patterns appear to be unique to individual species, at least within circumscribed geographic regions, and endemism is high, with isolated islands having unique suites of species that spatio-temporally sort out the signaling space with distinctive displays. Luminescent signaling in Caribbean ostracods is a rich field that promises to yield many new insights about signaling in the sea, mechanisms involved in manipulating the light production, and speciation and species relationships among the cypridinid ostracods (see e.g., Ellis & Oakley, 2016).

SUPPLEMENTARY MATERIAL

Supplementary material is available at *Journal of Crustacean Biology* online.

S1 Table. Families of Myodocopida.

S2 Video. Low-light level images of cypridinid courtship displays: lateral displays, plus vertical up and vertical down displays each from separate undescribed H-Group species in Belize, (**S2-A**), long vertical upward display from an undescribed H-Group species from Jamaica, (**S2-B**), and a shallow water vertical upward display from an undescribed *Kornickeria* species from Roatán, Honduras (**S2-C**).

S3 Video. NanoCT videos of sections through a male specimen of an undescribed H-Group cypridinid ostracod: cross (**S3-A**), longitudinal (**S3-B**), and horizontal (**S3-C**) sections.

ACKNOWLEDGEMENTS

This article is based on a keynote address given at the 18th International Symposium on Ostracoda, Santa Barbara, CA, USA on 29 August 2017. Many colleagues, graduate & undergraduate students, and friends have contributed to the studies of luminescent signaling ostracods with me. These include, in chronological order, Bill McFarland, Biff Bermingham, Myra Shulman, Andrea Huvard, Anne Cohen, Lou Kornicker, Margaret Kowalczyk, Lori Scanlon, Valerie King, Matthew Grober, Lisa Torres, Chris Coufal, Gretchen Gerrish, Trevor Rivers, Hilary Kates, Zeenat Patrawala, Evelyne Kuo, Martin Dohrn, Hilary Jeffkins, Elliot Lowndes, Jonathan Ruthven, Colleen Kearns, Mark Riccio, Lea Shulman, Todd Oakley, Niko Hensley, Emily Ellis, Nick Reda, and the Guna Yala nation of Panama, and numerous funding sources to me and my students including the National Science Foundation, National Geographic Society, and American Philosophical Society. I also thank an anonymous reviewer and the editor whose meticulous edits greatly improved the manuscript. I am especially grateful to my colleague and friend, Anne Cohen, a meticulous critic, but without whom much of this story would be very different.

REFERENCES

Abe, K., Ono, G., Yamada, K., Yamamura, N. & Ikuta, K. 2000. Multifunctions of the upper lip and a ventral reflecting organ in a bioluminescent ostracod *Vargula hilgendorfii* (Müller, 1890). *Hydrobiologia*, **419**: 73–82.

Anctil, M. 2018. *Luminous creatures: The history and science of light production in living organisms*. McGill-Queen's University Press, Montreal.

Anderson, R.S. 1935. Studies on bioluminescence, II. The partial purification of *Cypridina* luciferin. *Journal of General Physiology*, **19**: 301–305.

Angel, M.V. 1968. Bioluminescence in marine planktonic halocyprids ostracods. *Journal of the Marine Biological Association of the United Kingdom*, **48**: 255–257.

Baird, W. 1850. *The natural history of the British Entomostraca*. Ray Society, (Series 2, **Vol. 1**), London.

Baird, W. 1860. Note upon the genus *Cypridina* Milne-Edwards, with a description of some new species. *Proceedings of the Zoological Society of London*, **28**: 199–202.

Baker, H. 1753. *Employment for the microscope: In two parts*. R. Dodsley, London.

Brady, G.S. & Norman, A.M. 1896. A monograph of the marine and freshwater Ostracoda of the North Atlantic and of North-Western Europe. *The Scientific Transactions of the Royal Dublin Society*, **5**: 621–684.
Campbell, A.K. & Herring, P.J. 1990. Imidazolopyrazine bioluminescence in copepods and other marine organisms. *Marine Biology*, **104**: 219–225.
Coates, A.G. & Obando, J.A. 1996. The geological evolution of the Central American Isthmus. In: *Evolution and environment in tropical America* (J.B.S. Jackson, A.F. Budd & A.G. Coates, eds.), pp. 21–56. University of Chicago Press, Chicago.
Cohen, A.C. 1983. Rearing and postembryonic development of the myodocopid ostracode *Skogsbergia lerneri* from coral reefs of Belize and the Bahamas. *Journal of Crustacean Biology*, **3**: 235–256.
Cohen, A.C. & Morin, J.G. 1986. Three new luminescent ostracodes of the Genus *Vargula* (Myodocopida, Cypridinidae) from the San Blas region of Panama. *Contributions in Science, Natural History Museum of Los Angeles County*, **373**: 1–23.
Cohen, A.C. & Morin, J.G. 1989. Six new luminescent ostracodes of the genus *Vargula* (Myodocopida: Cypridinidae) from the San Blas region of Panama. *Journal of Crustacean Biology*, **9**: 297–340.
Cohen, A.C. & J.G. Morin. 1990. Morphological relationships of bioluminescent Caribbean species of *Vargula* (Myodocopa). In: *Ostracoda and global events* (R. Whatley & C. Maybury, eds.), pp. 381–400, Chapman and Hall, London.
Cohen, A.C. & J.G. Morin. 1993. The cypridinid copulatory limb and a new genus *Kornickeria* (Ostracoda: Myodocopida) and 4 new species of bioluminescent ostracodes from the Caribbean. *Zoological Journal of the Linnean Society*, **108**: 23–84.
Cohen, A.C. & Morin, J.G. 1997. External anatomy of the female genital (eighth) limbs and the setose openings in myodocopid ostracodes (Cypridinidae). *Acta Zoologica*, **78**: 85–96.
Cohen, A.C. & Morin, J.G. 2003. Sexual morphology, reproduction and the evolution of bioluminescence in Ostracoda. *Paleontological Society Papers*, **9**: 37–70.
Cohen, A.C. & Morin, J.G. 2010. Two new bioluminescent ostracode genera, *Enewton* and *Photeros* (Myodocopida: Cypridinidae), with three new species from Jamaica. *Journal of Crustacean Biology*, **30**: 1–55.
Conley, N. & Gerrish, G.A. 2019. Testing for multiple paternity in broods of the bioluminescent ostracod *Photeros annecohenae* (Torres & Morin, 2007) (Myodocopida: Cypridinidae). *Journal of Crustacean Biology*, **39**: 213–217.
Dahlgren, U. 1916. The production of light by animals. The luminous crustaceans. *Journal of the Franklin Institute*, **181**: 805–843.
Daniel, A. & Jothinayagam, J.T. 1979. Observations on nocturnal swarming of the planktonic ostracod *Cypridina dentata* (Müller) for mating in the northern Arabian Sea. *Bulletin of the Zoological Survey of India*, **2**: 25–28.
Doflein, F. 1906. Ueber Leuchtorgane bei Meerestieren. *Sitzungsberichte der Gesellschaft für Morphologie und Physiologie in München*, **22**: 133–136.
Ellis, E.A. & Oakley, T.O. 2016. High rates of species accumulation in animals with bioluminescent courtship displays. *Current Biology*, **26**: 1–6.
Gerrish, G.A. & Morin, J.G. 2008. Life cycle of a bioluminescent marine ostracode, *Vargula annecohenae* (Myodocopida: Cypridinidae). *Journal of Crustacean Biology*, **28**: 669–674.
Gerrish, G.A. & Morin, J.G. 2016. Living in sympatry via differentiation in time, space and display characters of courtship behaviors of bioluminescent marine ostracods. *Marine Biology*, **163**: 190–204.
Gerrish, G.A., Morin, J.G., Rivers, T.J. & Patrawala, Z. 2009. Darkness as an ecological resource: the role of light in partitioning the nocturnal niche. *Oecologia*, **160**: 525–536.
Godeheu de Riville. 1760. Mémoire sur la mer lumineuse. *Mémoires de l'Académie Royale des Sciences*, **3**: 269–276.
Godeheu de Riville. 1768. Observations on a luminous appearance of the sea, with a curious description of the minute animals, which occasion it; with the description of two other very singular sea animals. *Gentleman's Magazine*, **38**: 408 [English translation of 1760 article].
Haneda, Y. & Johnson, F.H. 1958. The luciferin-luciferase reaction in a fish, *Parapriacanthus beryciformes,* of newly discovered luminescence. *Proceedings of the National Academy of Sciences of the United States of America*, **44**: 127–129.
Haneda, Y., Johnson, F.H. & Shimomura, O. 1966. The origin of luciferin in the luminous ducts of *Parapriacanthus ransonneti, Pempheris klunzingeri*, and *Apogon ellioti*. In: *Bioluminescence in progress* (F.H. Johnson & Y. Haneda, eds.), pp. 533–545. Princeton University Press, Princeton, NJ, USA.
Harvey, E.N. 1916. The mechanisms of light production in animals. *Science*, **44**: 208–209.
Harvey, E.N. 1917. Studies on bioluminescence. IV. The chemistry of light production in a Japanese ostracod crustacean, *Cypridina hilgendorfii*, Müller. *American Journal of Physiology*, **42**: 318–341.
Harvey, E.N. 1952. *Bioluminescence*. Academic Press, New York.
Harvey, E.N. 1957. *A history of luminescence from the earliest times to 1900*. *Memoirs of the American Philosophical Society*, **44**: 1–692.
Hastings, J.W. & Morin, J.G. 1991. Bioluminescence. In: *Neural and integrative animal physiology* (C.L. Prosser, ed.), pp. 131–170, Wiley-Liss, New York.
Hensley, N.M., Ellis, E.A., Gerrish, G.A., Torres, E., Frawley, J.P., Oakley, T.H. & Rivers, T.J. 2019. Phenotypic evolution shaped by current enzyme function in the bioluminescent courtship signals of sea fireflies. *Proceedings of the Royal Society of London, B*, **286** [doi 20182621].
Herring, P.J. 1985. Bioluminescence in the Crustacea. *Journal of Crustacean Biology*, **5**: 557–573.
Horne, D.J., Schön, I., Smith, R.J. & Martens, K. 2005. What are Ostracoda? A cladistic analysis of the extant superfamilies of the subclasses Myodocopa and Podocopa (Crustacea: Ostracoda). In: *Crustacea and arthropod relationships* (S. Koenemann & R.A. Jenner, eds.). pp. 249–273. Taylor & Francis, Boca Raton, FL, USA.
Huvard, A.L. 1993. Structure of the light organ and immunocytochemical localization of luciferase in luminescent marine ostracods (Crustacea: Ostracoda: Cypridinidae). *Journal of Morphology*, **218**: 181–193.
Johnson, F.H. 1967. Edmund Newton Harvey 1887-1959. *Biographical Memoirs of the National Academy of Sciences*, **39**: 193–266.
Kelly, M.G. & Tett, P. 1978. Bioluminescence in the ocean. In: *Bioluminescence in action* (P.J. Herring, ed.), pp. 399–418. Academic Press, New York.
Kishi, Y., Goto, T., Hirata, Y., Shimomura, O. & Johnson, F.H. 1966a. *Cypridina* bioluminescence I: Structure of *Cypridina* luciferin. *Tetrahedron Letters*, **29**: 3427–3436.
Kishi, Y., Goto, T., Hirata, Y., Shimomura, O. & Johnson, F.H. 1966b. *Cypridina* bioluminescence II: Structural studies of *Cypridina* luciferin by means of a high resolution mass spectrometer and an amino acid analyzer. *Tetrahedron Letters*, **29**: 3437–3444.
Kishi, Y., Goto, T., Hirata, Y., Shimomura, O. & Johnson, F.H. 1966d. The structure of *Cypridina* luciferin. In: *Bioluminescence in progress* (F.H. Johnson & Y. Haneda, eds.). pp. 89–113, Princeton University Press, Princeton, N.J., USA.
Kishi, Y., Goto, T., Inoue, S., Sugiura, S. & Kishimoto, H. 1966c. *Cypridina* bioluminescence III. Total synthesis of *Cypridina* luciferin. *Tetrahedron Letters*, **29**: 3445–3450.
Kornicker, L.S. 1984. Cypridinidae of the continental shelves of southeastern North America, the northern Gulf of Mexico, and the West Indies (Ostracoda: Myodocopina). *Smithsonian Contributions to Zoology*, **401**: 1–37.
Kornicker, L.S. & Baker, J.H. 1977. *Vargula tsujii,* a new species of luminescent Ostracoda from Lower and Southern California (Myodocopa: Cypridinidae). *Proceedings of the Biological Society of Washington*, **90**: 218–231.
Kornicker, L.S. & King, C.E. 1965. A new species of luminescent Ostracoda from Jamaica, West Indies. *Micropaleontology*, **11**: 105–110.
Latreille, P.A. 1802. *Histoire naturelle, générale, et particulière des Crustacés et des Insectes*. **Vol. 3**. Dufart, Paris.
Latreille, P.A. 1806. *Genera Crustaceorum et Insectorum*. **Vol. 1**. Koenig, Paris.
Lessios, H.A. 2008. The great American schism: Divergence of marine organisms after the rise of the Central American Isthmus. *Annual Reviews of Ecology, Evolution and Systematics*, **39**: 63–91.
Linnaeus, C. 1758. *Systema Naturae per Regna Tria Naturae, Secundum Classes, Ordines, Genera, Species, cum Characteribus, Differentiis, Synonymis, Locis*. **Vol. 1**, Edn. 10. Reformata. Laurentii Salvii, Holmiae [= Stockholm].
Milne Edwards, H. 1834–1840. *Histoire naturelle des Crustacés, comprenant l'anatomie, la physiologie et la classification de ces animaux*. Vol. **III**. Librarie Encyclopédique de Roret, Paris.
Morin, J.G. 1983. Coastal bioluminescence: patterns and functions. *Bulletin of Marine Science*, **33**: 787–817.
Morin, J.G. 1986. "Firefleas" of the sea: luminescent signaling in marine ostracode crustaceans. *The Florida Entomologist*, **69**: 105–121.
Morin, J.G. 2011. Based on a review of the data, use of the term 'cypridinid' solves the *Cypridina/Vargula* dilemma for naming the constituents of the luminescent system of ostracods in the family Cypridinidae. *Luminescence*, **26**: 1–4.
Morin, J.G. & Bermingham, E.L. 1980. Bioluminescent patterns in a tropical ostracod. *American Zoologist*, **20**: 851.

Morin, J.G. & Cohen, A.C. 1988. Two new luminescent ostracodes of the genus *Vargula* (Myodocopida: Cypridinidae) from the San Blas region of Panama. *Journal of Crustacean Biology*, **8**: 620–638.

Morin, J.G. & Cohen, A.C. 1991. Bioluminescent displays, courtship, and reproduction in ostracodes. In: *Crustacean sexual biology* (R. Bauer & J. Martin, eds.), pp. 1–16. Columbia University Press, New York.

Morin, J.G. & Cohen, A.C. 2010. It's all about sex: bioluminescent courtship displays, morphological variation and sexual selection in two new genera of Caribbean ostracodes. *Journal of Crustacean Biology*, **30**: 56–67.

Morin, J.G. & Cohen, A.C. 2017. A guide to the morphology of bioluminescent signaling cypridinid ostracods from the Caribbean Sea, and a tabular key to the genera. *Zootaxa*, **4303**: 301–349.

Müller, G.W. 1890. Neue Cypridiniden. *Zoologische Jahrbücher*, **5**: 211–252.

Müller, G.W. 1906a. Ostracoda. *Wissenschaftliche Ergebnisse der Deutschen Tiefsee-Expedition auf dem Dampfer "Valdivia" 1898–1899*, **8**: 27–154.

Müller, G.W. 1906b. Die Ostracoden der Siboga-Expedition. *Siboga Expeditie Monographie*, **30**: 1–67.

Müller, G.W. 1912. Ostracoda. *Das Tierreich*, **31**: 1–434.

Müller, O.F. 1776. *Zoolgiae Danicae prodromus, seu animalium Daniae et Norvegiae indigenarum characters, nomina, et synonyma imprimis polularium*. Hallageriis, London.

Müller, O.F. 1785. *Entomostraca seu Insecta Testacea*. Sumtibus J. G. Mülleriani, London.

Oakley, T.H. 2005. Myodocopa (Crustacea: Ostracoda) as models for evolutionary studies of and vision: multiple origins of bioluminescence and extreme sexual dimorphism. *Hydrobiologia*, **538**: 179–192.

Oakley, T.H., Wolfe, J.M., Lindgren, R. & Zaharoff, A.K. 2012. Phylotranscriptomics to bring the understudied into the fold: Monophyletic Ostracoda, fossil placement, and pancrustacean phylogeny. *Molecular Biology and Evolution*, **30**: 215–233.

O'Dea, A., Lessios, H.A., Coates, A.G., Eytan, R.I., Restrepo-Moreno, S.A., Cione, A.L., Collins, L.S., de Queiroz, A., Farris, D.W., Norris, R.D., Stallard, R.F., Woodburne, M.O., Aguilera, O., Aubry, M.P., Berggren, W.A., Budd, A.F., Cozzuol, M.A., Coppard, S.E., Duque-Caro, H., Finnegan, S., Gasparini, G.M., Grossman, E.L., Johnson, K.G., Keigwin, L.D., Knowlton, N., Leigh, E.G., Leonard-Pingel, J.S., Marko, P.B., Pyenson, N.D., Rachello-Dolmen, P.G., Soibelzon, E., Soibelzon, L., Todd, J.A., Vermeij, G.J., Jackson, J.B.C. 2016. Formation of the Isthmus of Panama. *Science Advances*, **2**: e1600883 [doi: 10.1126/sciadv.1600883].

Parker, A.R. 1995. Discovery of functional iridescence and its coevolution with eyes in the phylogeny of Ostracoda (Crustacea). *Proceedings of the Royal Society of London, B*, **262**: 349–355.

Poulsen, E.M. 1962. Ostracoda-Myodocopa.1: Cypridiniformes-Cypridinidae. *Dana Report*, **57**: 1–414.

Regier, J.C., Shultz, J.W., Zwick, A., Hussey, A., Ball, B., Wetzer, R., Martin, J.W. & Cunningham, C.W. 2010. Arthropod relationships revealed by phylogenomic analysis of nuclear protein-coding sequences. *Nature*, **463**: 1079–1083.

Rivers, T.J. & Morin, J.G. 2008. Complex sexual courtship displays by luminescent male marine ostracods. *Journal of Experimental Biology*, **211**: 2252–2262.

Rivers, T.J. & Morin, J.G. 2009. Plasticity of male mating behavior in a marine bioluminescent ostracod in both time and space. *Animal Behaviour*, **78**: 723–734.

Rivers, T.J. & Morin, J.G. 2012. The relative cost of using luminescence for sex and defense: light budgets in cypridinid ostracods. *Journal of Experimental Biology*, **215**: 2860–2868 [with cover photo].

Rivers, T.J. & Morin, J.G. 2013. Female ostracods respond to and intercept artificial conspecific male luminescent courtship displays. *Behavioral Ecology*, **24**: 877–887.

Sars, G.O. 1866. Oversigt af Norges marine Ostracoder. *Forhandlinger Videnskabs-Selskabet Christiania*, **7**: 1–130.

Shimomura, O. 2006. *Bioluminescence: chemical principles and methods*. World Scientific Publishing, Singapore.

Shimomura, O., Goto, T. & Hirata, Y. 1957. Crystalline *Cypridina* luciferin. *Bulletin of the Chemical Society of Japan*, **30**: 929–933.

Shimomura, O., Johnson, F.H. & Saiga, Y. 1961. Purification and properties of *Cypridina* luciferase. *Journal of Cellular and Comparative Physiology*, **58**: 113–123.

Shimomura, O., Shimomura, S. & Brinegar, J.H. 2018. *Luminous pursuit: jellyfish, GFP, and the unforseen path to the Nobel Prize*. World Scientific Publishing, Singapore.

Skogsberg, T. 1920. Studies on marine ostracods, I: Cypridinids, halocyprids and polycopids. *Zoologiska Bidrag från Uppsala*, Supplement, **1**: 1–784.

Thompson, E.M., Nafpaktitis, B.G. & Tsuji, F.I. 1987. Induction of bioluminescence in the marine fish, *Porichthys,* by *Vargula* (crustacean) luciferin. Evidence for *de novo* synthesis or recycling of luciferin. *Photochemistry and Photobiology*, **45**: 529–533.

Thompson, E.M., Nagata S. & Tsuji, F.I. 1989. Cloning and expression of cDNA for the luciferase from the marine ostracod *Vargula hilgendorfii*. *Proceedings of the National Academy of Sciences of the United States of America*, **86**: 6567–6571.

Torres, E. & Cohen, A.C. 2005. *Vargula morini*, a new species of bioluminescent ostracode (Myodocopida: Cypridinidae) from Belize and an associated copepod (Copepoda: Siphonostomatoida: Nicothoidae). *Journal of Crustacean Biology*, **25**: 11–24.

Torres, E. & Gonzalez, E.L. 2007. Molecular phylogeny of cypridinid ostracodes and the evolution of bioluminescence. In: *Proceedings of the 14th International Symposium on Bioluminescence and Chemiluminescence: Chemistry, Biology and Applications* (A.A. Szalay, P.J. Hill, L.J. Kricka & P.E. Stanley, eds.). pp. 269–272. World Scientific Publishing Company, Singapore.

Torres, E. & Morin, J.G. 2007. *Vargula annecohenae*, a new species of bioluminescent ostracode (Myodocopida: Cypridinidae) from Belize. *Journal of Crustacean Biology*, **27**: 649–659.

Tsuji, F.I. & Sowinski, R. 1961. Purification and molecular weight of *Cypridina* luciferase. *Journal of Cellular and Comparative Physiology*, **58**: 125–130.

Tsuji, F.I., Barnes, A.T. & Case, J.F. 1972. Bioluminescence in the marine teleost, *Porichthys notatus*, and its induction in a non-luminescent form by *Cypridina* (ostracod) luciferin. *Nature*, **237**: 515–516.

Tsuji, F.I., Lynch, R.V. & Stevens, C.L. 1974. Some properties of luciferase from the bioluminescent crustacean *Cypridina hilgendorfii*. *Biochemistry*, **13**: 5204–5209.

Watanabe, H. 1897. The phosphorescence of *Cypridina hilgendorfii*. *Annotationes Zoologicae Japonenses*, **1**: 69–70.

Wilson, C.B. 1913. Crustacean parasites of West Indian fishes and land crabs, with descriptions of new genera and species. *Proceedings of the United States National Museum*, **44**: 189–277.

Wilson, T. & Hastings, J.W. 2013. *Bioluminescence: Living lights, lights for living*. Harvard University Press, Cambridge, MA, USA.