Laboratory breeding and rearing of cellar spider, *Crossopriza lyoni* Blackwall

Johan Ariff Mohtar 1 · Mohd Faidz Mohamad Shahimin 1

Received: 16 May 2022 / Accepted: 20 September 2022 / Published online: 3 October 2022
© The Author(s), under exclusive licence to Springer-Verlag GmbH Germany, part of Springer Nature 2022

Abstract

Spiders have emerged as one of the leading model organisms in many research fields due to their compelling biology. Often, scientific investigations involving the use of spiders face inevitable problems associated with the lack of specimens from laboratory stock, resulting in difficulties in yielding reproducible investigations for predictive research. Thus, several species of well-studied spiders, including *Parasteatoda tepidariorum*, have been successfully bred for such purposes. *Crossopriza lyoni* is a Haplogyne spider, globally distributed and widespread in human inhabitants, prompting interest in various studies over the last decades. Despite its scientific importance, no laboratory-bred *C. lyoni* has been documented. Therefore, we describe a successful captive breeding system of the species under controlled conditions to establish a laboratory stock culture. Methods for mating induction, egg collection and segregation, artificial embryo incubation, and colony husbandry are discussed. The technique presented is a simple and low-cost approach that is reliable for *C. lyoni* propagation in the laboratory over several generations.

Keywords Spider · Laboratory stock · *Crossopriza lyoni* · Haplogyne · Breeding · Propagation

Introduction

Spiders have contributed to many scientific breakthroughs in the studies of evolutionary development, behaviour, ecology, venomology, and silkomics, implying spiders’ equally vital contributions to arthropods studies such as insects. However, scientists who work with spiders have limited access to laboratory colonies, and the only available option is to use wild specimens. The lack of spider laboratory colonies is due to their laborious mass propagation, specifically their cannibalistic behavior and long life cycle. Limitation in the laboratory colony often impedes reliable scientific investigations for predictive research. At present, only a few spider species were thoroughly investigated, including *Parasteatoda tepidariorum* (Kanayama et al. 2010; Pechmann 2016; Oda and Akiyama-Oda 2020), *Cupiennius salei* (McConney et al. 2009), *Pholcus phalangiodes* (Turetzek and Prpic 2016), and *Trichonephila edulis* (Liebsch et al. 2020). These species have been extensively bred for laboratory stock establishment over many generations.

For the past 90 years, *C. lyoni*, a non-orb weaver species (commonly known as cellar spider), has been used as a research subject in cytology (Nath 1928; Nath and Dawan 1955; Nath et al. 1958; Shaima et al. 1959; Sareen 1965; Prakash 2014), physiology (Shunmugavelu and Palanichamy 1993), predatory behavior (Nandi and Raut 1986; Shunmugavelu and Palanichamy 1995; Strickman et al. 1997), chemical biology (Maya et al. 1982), genetics (Oliveira et al. 2007; Prakash 2015), and silk studies (Karuppaswamy et al. 1984a, b; Mohtar et al. 2018; Yeng et al. 2018). Despite its prominent significance, no *C. lyoni* laboratory colony has ever been reported due to the lack of its rearing and breeding methods. Thus, there is a need to propagate laboratory colonies of *C. lyoni* to further shed some insights into spiders’ diversity via morphological and genetic studies. Moreover, as it is not yet evaluated as an endangered species by the International Union for Conservation of Nature (IUCN) (Roy et al. 2019), working with the species is not subjected to any legislative act. In this paper, we describe a successful system for keeping *C. lyoni* under laboratory-controlled conditions for scientific purposes at the Tissue Culture and
Biomolecular Laboratory (TCB), Faculty of Chemical Engineering Technology, Universiti Malaysia Perlis. The method outlines five major topics: mating induction, egg oviposition and harvesting, egg mass separation, artificial incubation of embryos, and spider husbandry.

Cellar spider, Crossopriza lyoni

*C. lyoni* Blackwall, 1867 (Araneae: Pholcidae) is a cosmopolitan spider that belongs to the basal clade Haplogynae. Its geographical distribution mostly spans the tropical to subtropical regions (WSC 2022), and in Malaysia, it lives synanthropically in human dwellings (Dzulhelmi and Suriyanti 2015; Nik and Suhaila 2019; Koh and Bay 2019). Surprisingly, it has also been discovered to thrive in temperate regions of many European countries and America, implying its high adaptability to various environmental conditions (Edwards 1993; Bauer et al. 2016). Consequently, *C. lyoni* has been currently catalogued as an invasive species (CABI 2019).

Morphologically, the spider has a recognizable box shape and angular opisthosoma (abdomen), with a cone-like protrusion on the upper back. From the lateral view, the opisthosoma has a flattened, triangular appearance, with the caudal end tapering to a point (Fig. 1a). Occasionally, white lateral stripes are observable, covering the entire abdomen. A brown dorsal median indentation is visible on the carapace of its subcircular cephalothorax (Fig. 1b). With a miniscule body size between 3 and 7 mm and 2.5 and 6 mm for females and males, both possess extraordinary long, slender legs. They are covered with numerous tiny longitudinal brown spots and white stripes coating the ends of the femur and tibia. Despite similar body measurements, *C. lyoni* exhibits sexual dimorphism, where males have slightly longer front leg pairs than females. Therefore, they are often mistaken for a daddy longlegs, where the correct term should be the harvestman (Opiliones) (Berenbaum 2004).

Generally, *C. lyoni* constructs a loosely, irregular web commonly spotted in human residences, such as cellars, where its name is commonly dubbed. The high number of spiders in a place renders them pestiferous due to the extensive webbings. Members of the pholcid family are known to adopt a facultative life cycle, where spiders of all stages can be found both in solitary and in one large communal web. Although group-living behavior is prominent in pholcids, the degree of sociality varies. For instance, *Holocnemus pluchei* was reported to share an interconnected large web with dozens of individuals of different stages and gender (Jakob et al. 2000). This mass aggregation of pholcids results from the crowding of spiders in small habitat patches. As the web becomes crowded with huge individual numbers, where competition for prey, reproduction, and survival are high, spiders seek to build solitary webs at a suitable site.

In Malaysia, a group-living *Pholcus* species was discovered in mass containing all developmental stages of spiderlings and adults in the tropical rainforest. Their sociality is regarded as communally behaved, with web repair and defence but competitively aggressive in prey capture (Endo and Mohamed 2001). Despite the lack of systematic studies on the web living behavior of *C. lyoni*, direct observations from the public suggest that it follows the same manner of facultative web life (B. Russell, pers. comm., September 6, 2019). Not much is known about the enemies of *C. lyoni*, but when disturbed, the spider poses vigorous body vibration as a defence warning (Strickman et al. 1997; Huber 2014). The spider is an extremely efficient hunter of mosquitoes and has been proposed to be used in an integrated mosquito population control to reduce dengue transmission. Many pholcid species, including *C. lyoni*, have never been reported to inflict an appreciable bite on humans despite having an amount of venom. The toxins are used to paralyze their prey while feeding and are completely harmless to humans (Berenbaum 2004; Gupta and Upadhayay 2018; Zobel-Thropp et al. 2019).

Spider sampling and induction of mating

At TCB, the laboratory stocks of *C. lyoni* have been propagated for 7 generations and 1201 days. It was initiated from two pairs of spiders randomly collected from the web in

---

**Fig. 1** a An adult female of *C. lyoni* showing the white bands girdling the ends of the femur and tibiae of the legs. b Stereoscopic view of the brown median indentation on the cephalothorax with its DNA barcode below.

© Springer
an abandoned storehouse staircase area (6°39′1.25″N, 100°15′29.46″E) (Fig. 2a). Using a cobweb duster, a male and female pair were caught and placed in a cylindrical transparent container (7.8 × 14 cm) with holes for ventilation, as shown in Fig. 2b. The enclosure is flipped, and holes are drilled on the top and upper part of the wall for ventilation and food insertion. Although bigger containers of different sizes and shapes can also accommodate the spider pair, we found that a cylindrical enclosure with similar measurements is the most suitable as it provides ample space for the spider to make contact for mating within a short time and construct more densely irregular web. These spiders were maintained at room temperature between 28 and 30 °C, with humidity ranging from 58 to 82% and a 12-h photophase:12-h scotophase.

After captivation, we observed that the male C. lyoni rarely attached exploration threads (equivalent to dragline silk) while walking on bare surfaces when placed singly in a container. The male remained on the surface even after a day in the container, without the silken web. Our observation conforms with previous studies, which reported less web building in adult male pholcids compared to females (Eberhard and Briceño 1985; Li and Kuan 2006) and male pholcids rarely leave trail of dragline silk upon walking on a substrate for safety (Wolff et al. 2017). Because the male spider does not produce any web, a female spider was used to induce web building by inverting the container upside down. The female C. lyoni immediately started to spin exploration thread (dragline silk) as it moved around the surface. After overnight, the enclosure was flipped with its lid at the bottom, allowing the spider to hang over the threads. The female kept expanding the loosely tangled web until a “dome-sheet” structure was achieved, as reported by Hajer and Řeháková (2003).

In general, C. lyoni constructs a mesh of irregular, stereotypical dome-shaped sheet web with spotted white puffs where the spider hangs upside down at the apex of the dome; a typical characteristic of many pholcid webs (Hajer and Řeháková 2003; Escalante 2013; Huber 2014) (Fig. 2a). Once a site is selected, pholcids initially attach exploration threads to the substrate for several days prior to web building (Escalante 2013). The web is then constructed by laying thick, stretched skeleton threads containing a bundle of fibrils with a diameter ranging from 5 to 6 µm, with each fibril approximately 1.2 µm (Escalante 2013). These smooth threads are believed to be equivalent to the major ampullate silk that serves as scaffolding or framework of the web. It is noteworthy that the exploration and skeleton threads may probably be referred to as the same fiber by the author. Within the network space of stretched threads, thinner filling silks comprising invariably a single pair of fibrils are spun (Escalante 2013). These threads are thinner than the skeleton fiber, with each fibril having a diameter from 0.8 to 0.9 µm (Escalante 2013). The filling threads act as web reinforcement and are postulated to be equivalent to the minor ampullate silk.

Our captive female C. lyoni kept adding new exploration threads to the existing web even after it was completed, similar to other pholcids (Andrés 2011; Escalante 2013). This results in the growth of a large, interconnected silk mesh inside the container. Additionally, cottony flecks were produced on the web for protection against predators (Hajer and Řeháková 2003) as early as in the first instar of C. lyoni. This feature was also associated with pre-moulting and ovisposition (Strickman et al. 1997). The flecks are made largely from unstressed fibrils by breaking off the stretched skeleton threads (Hajer and Řeháková 2003).

We introduced the male C. lyoni inside the container to induce mating when the mesh became slightly thicker. Prior to transferring the male, the female was first fed a medium-sized mealworm to prevent the male from being eaten alive. When prey is entangled on the web, C. lyoni throws silk over it and loosely wraps it without rotation using its hind legs before biting on the soft spot of the prey (Fig. 3a). Copulation with the female was induced after transfer. As early as 5 min upon contact, the mating ritual was observed when the male started to drum its opisthosoma back and forth in front of the female. We postulate that this courtship behavior is to attract the female, while the abdominal movement might have released a specific pheromone or acted as a stridulation to produce low-frequency copulating sound. Nevertheless, Maya et al. (1982) reported that adult females of C. lyoni were responsible for synthesizing an aerial contact sex pheromone when a male is sighted, and the male abdominal vibration may be a sign of excitation upon contact with the chemical. Moreover, the abdominal gesture may also generate specific mating vibrations sent through the mesh network.

![Fig. 2](image-url) **a** A typical irregular dome-shaped sheet web of an adult spider at a corner of an abandoned staircase. **b** The plastic enclosure used to accommodate adult spiders in the laboratory.
to lure the female’s attention. While shaking its abdomen, the male reached the female by its two frontal legs and drew her toward him.

Once accepted, the male spider positioned itself on the female’s underside and inserted the enlarged palpal bulbs into the genital orifice (epigynum), a typical mating means in pholcid spiders (Eberhard and Huber 2010) (Fig. 3b). The copulation was observed to last for approximately 40 to 60 min, similar to other pholcids (Huber and Eberhard 1997). The mating continued several times for 10.0 ± 0.71 consecutive days before egg deposition. Throughout the mating period, mealworms were perpetually offered to provide nutrition to the spiders for sperm and egg development, promote denser web building, and, most importantly, inhibit cannibalism. Surprisingly, male and female *C. lyoni* often displayed communal feeding behavior, a trait commonly seen in social spiders, although prey capture was performed individually (Fig. 3c).

**Species identification and verification**

In order to confirm the collected spiders belong to the correct species, the pairs were initially subjected to morphological diagnosis in reference to Koh and Bay (2019) and Nentwig et al. (2022). The characteristics indicated that of an individual of *C. lyoni*. In addition, we also adopted the DNA barcoding analysis to further verify the species using the cytochrome oxidase subunit I (COI) gene, according to Rowan and Paul (2005). A set of primers were designed and synthesised: forward primer LCO1490A (5′- GGT CAACAAATCATAAGATATTGG-3′); two reverse primers, chelicerae reverse 1 (CR1, 5′- CCTCCTCCT GAAGGGTCAAAAAATGA-3′), and chelicerae reverse 2 (CR2, 5′- GGATGGCCAAAAATCAAATAAA TG-3′). The result of polymerase chain reaction (PCR)-based amplification using the genomic DNA of *C. lyoni* extracted from the legs (*N* = 2) showed that the trimmed LCO1490A/CR2-amplified COI gene (650 bp), displayed a 99.69% homology (100% query coverage, *E*-value = 0.0) to *C. lyoni* in the BLASTn search using the National Centre for Biotechnology Information (NCBI) database (Koay 2018). The COI gene sequence was deposited in the GenBank under the accession number ON552547.

**Egg oviposition**

After continuous mating, the female started to lay eggs. Each embryo was deposited in the sticky liquid into a ball mass (Fig. 4a). The glutinous liquid is equivalent to spumaline used to cover insect egg mass, which absorbs water to prevent eggs from desiccation. When the egg sac hardened after a few minutes, the female wrapped it with loosely silk threads before grasping it by its chelicerae (Fig. 4b). Interestingly, the egg deposition period of *C. lyoni* in our captivity was twofold longer than that of Strickman et al. (1997). It is probably due to the slight regional temperature and humidity differences. Occasionally, the egg sac was temporarily hung on the web when the female started to feed. Subsequent copulations still occurred even when the female was holding the eggs (Fig. 4c). This brood care plays a significant role in the successful evolution of pholcid to reproduce without the need for extensive protective egg case silks (Huber 2014). The two pairs produced a total of 133 eggs, which vary in each generation. They were spherical and greenish upon deposition but gradually turned brownish after a few hours. Their size is approximately 0.90 ± 0.04 mm (Fig. 4d).

**Egg harvesting and mass separation**

Considering the nature of the egg mass, a proper technique for sac collection was developed based on the water separation technique known as the water droplet (WD) technique. This technique allows for subtle embryo separation with little to no damage infliction. Although spider silk fibers generally display supercontraction property, where it contracts from its original size when exposed to...
water molecules to maintain an amount of stress that helps prevent the web from sagging and collapsing (Agnarsson et al. 2009), pholcids’ silks exhibit low supercontraction property due to the lack of the well-differentiated spidroin-1 and spidroin-2 types (Boutry and Blackledge 2010). Hence, the WD technique is advantageous in separating individual eggs of *C. lyoni*.

By firmly holding the female by its legs, the cephalothorax was dipped into a water droplet to allow her to lose the grip so that the egg sac was detached from its chelicerae, using a fine wet brush with a narrow tip (Fig. 5a–d). It

---

**Fig. 4** a An up-close of the egg sac showing the loosely wrapping silk with denser threads (black arrow) for female to hold at its chelicerae. b An egg mass held by an adult female. c Post-oviposition mating by a female with its egg mass. d Individual eggs are separated after being immersed in water droplet and the egg size measurement is as indicated by the eggs with white double-headed arrows.
was soaked in the water droplet for 1 min to allow water absorption of the wrapping threads (Fig. 5e). Due to the low supercontraction, the wrapping silks immensely shrunk. The sac was gently poked against the water droplet using the brush to detangle the wrapping silks, releasing the individual eggs into the droplet (Fig. 5f). Because of the low adhesive property of the polysaccharide dope covering the egg mass upon soaking in water, the eggs were easily separated. However, we found that dipping embryos in water for too long inflicted damage to them due to the softening of the chorion layer.

After complete detachment, the eggs were picked immediately using a clean brush and transferred into a sterile container with a dimension of $144 \times 95 \times 43$ (L x W x H) mm (Fig. 5g). The individual embryo was held at the tip of the brush and gently patted against a dry tissue to avoid harsh stroke that could result in high embryo mortality. Nevertheless, we discovered that if an intact egg sac accidentally fell to the ground, relatively high numbers of the eggs could still survive and hatch. Prior to incubation, the eggs were completely dried at room temperature before incubation and promoted fungal growth inside the rearing container.

**Artificial incubation of spider embryo**

In the laboratory, hatching on the web typically occurs after 19 to 20 days at 28 to 30 °C, with humidity ranging from 58 to 82% and a 12-h photophase:12-h scotophase. The spiderlings (prenymphs) partially emerged from the eggs within 24 h before leaving the sac (Fig. 6a). However, our observation contradicted Strickman et al. (1997), who reported the emergence of the prenymphs at 11 to 13 days after oviposition at 30 °C, with a 15-h of photophase:15-h scotophase. Our contradicting observation could be due to differences in the environmental condition or other yet-to-be uncovered factors. Spiderlings were inactive for the first 4 days and remained on the web with their mother. At this stage, they did not feed but freely spun dragline silks while dispersing. We have optimized the conditions for artificial embryo incubation at 29 °C with 73% humidity. They underwent an average developmental period of 19 days in this controlled state, with a hatching rate reaching 89.5% (Fig. 6b). The temperature and humidity regime values simulated the field conditions (a representative condition of the laboratory), where the embryos would have developed and hatched on the web with their mother. Throughout this incubation period, the embryos were periodically monitored to avoid mite infestation and mould growth in the rearing container (Fig. 6c).

**Laboratory husbandry**

**Post-embryonic development to adulthood**

*C. lyoni* undergoes a series of post-embryonic developmental stages before reaching maturity. Within 24 h after eclosion in the incubator, the newly hatched spiderlings ($N=119$) stayed dormant near the eggshell for a few minutes prior to displacement. The newly hatched spiderlings are known as prenymphs, according to the terminology by Platel (1989) in his study on *Pholcus phalangioides*. They emerged either in the early morning or in the late evening. Once active, the prenymphs moved toward the top of the container as young spiders possess an innate negative geotropism. During the displacement, individual prenymphs were observed to lay dragline threads fixed to the tissue substrate in a discrete pattern, as reported by Vollrath (1999). After a few hours, a mesh of irregular threads was constructed, with all prenymphs assembled in mass, which is a common trait observed in many solitary species in the field prior to dispersal, known as a transient gregarious phase (Fig. 7a).

We also observed the collective displacement in the prenymphs that hatched on the web with their mother, although the construction of the gossamer mesh was not
obvious due to the presence of adult silken web. This gossamer mesh was a collective thread of dragline silk from different individuals used later as skeleton threads to construct the framework of the irregular dome-sheet web by the adults (Fig. 7b). According to Jeanson et al. (2004), dragline attachment induces spiderling aggregation of a solitary species, which explains the construction of the gossamer mesh by the prenymphs. When one juvenile moved, it randomly selected the left or right direction. Due to the discrete attachment pattern of dragline silk, the juvenile occasionally created a shortcut that increased the probability of a second spiderling taking the route and continued by the next spiderlings. Hence, each prenymph contributed to the growth of the webbing structure, and ultimately, all individuals were randomly distributed on the mesh. Moreover, the growth of the mesh can also be explained by the affinity for silk laid by conspecifics that led to the web aggregation (Hodge and Storfer-Isser 1997; Schuck-Paim and Alonso 2001).

All prenymphs of C. lyoni were inactive on the gossamer mesh for the next 72 h, and no feeding activity occurred. Individual separation was not performed during this phase since an early study by Hajer and Řeháková (2003) reported that early segregation of prenymphs caused unknown death after the first moult. However, when separation was performed in the same manner after the first ecdysis, spiderlings readily constructed a network of webs and thrived (Hajer and Řeháková 2003). After 3 days of egg hatching, the C. lyoni prenymphs underwent their first moulting. Each first instar spiderling from this stage was separated into a single Drosophila vial using a fine brush to avoid cannibalism (Fig. 7c). Feeding was performed within 24 h after a moult. Insects were mainly used as feeders for the spider brood. However, although C. lyoni is not a fussy eater and can be fed with various live insects, they rarely consume dead insects on the web. Four types of insects were utilized at TCB: sugar ant (Paratrechina longicornis), fruit fly (Drosophila melanogaster), scuttle fly (Megaselia sp.), and mealworm (Tenebrio molitor). P. longicornis was administered as an initial feeder insect for the first instar spiderling. Although D. melanogaster could be fed, it was rarely consumed due to its relatively larger size that often damaged the sheet web as the fly wriggled when entangled in the mesh. All spiderlings were kept until adulthood at 30 °C with 77% humidity in complete darkness as pholcid spider growth was not affected by photoperiod (Miyashita 1988) (Fig. 7d).

The spiderlings (N = 109) spent an average time of 22.23 ± 0.42 days in the first instar. The feeding activity was ceased for several days before entering the subsequent instar to allow spatiotemporal preparation for ecdysis (Vetter and Rust 2010). The cottony flecks were heavily woven in the web at this pre-moult stage. In the second instar, the spiderlings (N = 109) thrived for 39.75 ± 0.67 days on average, where they were fed with fruit flies or phorid flies. Prior to feeding, the flies were subjected to anesthesia at 4 °C for 5 min and immediately loaded into the vial. Following another four consecutive moults, the spiderlings developed from the third to the fifth instar with the mean days of 59.53 ± 0.99 days (N = 109), 83.82 ± 1.31 days (N = 108),
and \(114.23 \pm 1.57\) days \((N = 99)\), before sexually mature. However, the developmental time and maturity varied in each generation.

Maturity normally commenced after the sixth moult at a higher frequency since our laboratory colonies were exclusively fed with medium to large mealworms from the fourth instar until the adult stage. At this point, the web became denser, and it could retain large prey for feeding. Spiders are excellent hunters to catch prey larger than themselves. Interestingly, the prenymphs of \textit{C. lyoni} are even known to overpower prey four times their size (Strickman et al. 1997). However, in several generations, we also noticed that few individuals underwent an additional seventh moult despite sufficient high-quality food intake. Surprisingly, a small number of spiderlings matured as early as the fourth and fifth mouls, and a similar maturity pattern has also been observed in \textit{H. pluchei} when fed with high food levels (Jakob and Dingle 1990). All spiderlings were fed routinely 4 to 5 times a week.

In terms of the offspring sex ratio, the percentage number of females was 80\% higher than the males, although it varied in each generation. Male and female spiders can be easily discerned in the ultimate nymphaal instar (subadult) prior to maturation by their genital organs. Many spiders undergo notable sexual organ development in the final nymphaal stage (Quade et al. 2019; Cordellier et al. 2020). In female subadults, a small hump is obvious at the base of opisthosa on the ventral side (Fig. 8a and b). This protrusion developed into a functional epigynum that received the male genital bulbs during mating (Fig. 8c and d). Biaggio et al. (2016) indicated that the female epigynum is visible below the cuticle prior to ultimate moult in some spiders and the genital structures lack the nerve and sense organs (Eberhard and Huber 1998b). However, male subadults of \textit{C. lyoni} developed a pair of elaborate extensions of the pedipalp segments, bearing the genital bulbs. In the early subadult instar, the pedipalps were preceded by a pair of small appendages.
but gradually increased in size (Fig. 8e). After the ultimate moult, the bulbs were readily utilized for sperm transfer in matured males (Eberhard and Huber 1998b) (Fig. 8f). These morphological characteristics can be used as a visible indicator to determine the gender of C. lyoni spiderling at the final instar.

Although the development of sexual organs is largely prominent in the subadult stage, it can commence as early as in the pre-subadult stage in a few male and female species (Mahmoudi et al. 2008; Biaggio et al. 2016; Quade et al. 2019). Nevertheless, such a trait was not observable in the pre-subadults of C. lyoni through the naked eyes. Following the ultimate moult, adult spiders were transferred into the cylindrical container (7.8 × 14 cm) for mating, as previously described. They were routinely fed with large-sized mealworms. After feeding, they usually drop the prey remains from the web, which were pulled from the web using forceps if entangled. Feces appeared as white tarry drops at the bottom of the enclosure and were frequently cleaned before feeding to impede mould growth and prevent mite infestation. Copulation could be induced 48 h after the final moult, whereby they were initially fed before pairing the male with the female. In captivity, both male and female C. lyoni exhibited a comparable number of molts per generation.

In the laboratory, a monogamous mating system was adopted to establish a new generation of spiders, although Strickman et al. (1997) reported that a single male of C. lyoni could mate with multiple females at one time, and a single female produced eggs up to six batches. The frequency of egg deposition by a single female in our captivity varied from two to nine times with the same male. This is congruent with previous studies on pholcids (Miyashita 1988; Platel 1989; Jakob and Dingle 1990; Turetzek and Prpic 2016). A wild-caught female of C. lyoni was reported to display longevity for at least 194 days (Strickman et al. 1997). Although we did not accurately estimate longevity for all individual spiders, we observed that most females had lived as long as 425 days after maturity. Interestingly, the only surviving female from the first generation had lived for at least 769 days. Male spiders usually die soon after mating or are consumed by females during copulation. Throughout the rearing period, no bites from the adult spiders have been inflicted during handling.

**Conclusion**

The initiation of laboratory-reared populations aims to obtain a culture that reflects the variations observed in nature. If the variation is undesirable, one can further develop specific strains from the established culture to suit the needs of an experiment through inbreeding, mutation, or genetic modification. Crossopriza lyoni is easily reared and propagated from egg to adult stage under laboratory conditions rapidly, as it can mature as early as the fourth instar. A shorter development period readily provides evolutionary advantages for spiders and scientists perceived it as a manipulative trait because their long-life cycle hampers rapid rearing in large numbers for genetic crosses. A simple, low-cost captive breeding system comprising five important steps from mating induction to laboratory husbandry has been successfully established to generate laboratory stocks. Although the methods described in this paper provide a foundation for rearing a single pholcid species, they can be further modified for improvement on other spiders. One of the highlights of the system is the recovery of a single egg that can be directly used in numerous experiments such as microinjection. Given its small body size, high adaptability, longevity, shorter development time, high reproductive success, and harmlessness, C. lyoni readily provides a great opportunity to be extensively explored as the next non-model spider for future scientific studies.

**Acknowledgements** We would like to thank the Faculty of Chemical Engineering Technology, Universiti Malaysia Perlis (UniMAP), for the use of laboratory facilities. Lastly, our heartfelt gratitude goes to Mr. Mohd Mushahril Abdul Shukor for the professional macro photographic images.

**Author contribution** Conceptualization: Johan Ariff Mohtar; methodology: Johan Ariff Mohtar and Mohd Faidz Mohamad Shahimin; formal analysis and investigation: Johan Ariff Mohtar and Mohd Faidz Mohamad Shahimin; writing—original draft preparation: Johan Ariff Mohtar; writing—review and editing: Mohd Faidz Mohamad Shahimin and Johan Ariff Mohtar; funding acquisition: Johan Ariff Mohtar and Mohd Faidz Mohamad Shahimin; resources: Johan Ariff Mohtar and Mohd Faidz Mohamad Shahimin.

**Funding** This work was funded by the Fundamental Research Grant Scheme (FRGS) under the grant number FRGS/1/2017/STG05/UNIMAP/03/1 from the Ministry of Education Malaysia.

**Data availability** All data generated or analyzed during this study are provided in the text and figures.

**Code availability** Not applicable.

**Declarations**

**Competing interests** The authors declare no competing interests.

**Ethics approval and consent to participate** Not applicable.

**Consent for publication** Not applicable.

**Conflicts of interest** The authors declare no competing interests.
Schuck-Paim C, Alonso WJ (2001) Deciding where to settle: conspecific attraction and web site selection in the orb-web spider Nephilengys cruentata. Anim Behav 62(5):1007–1012. https://doi.org/10.1006/anbe.2001.1841

Shaima GP, Gupta BL, Farehah R (1959) Cytological studies on the Indian spiders. III. An analysis of the chromosomes in the male germ cells of the spider, Crossopriza lyoni (Blackwall), fam. Pholcidae. Res Bull Panjab Univ Sci 10:49–53

Shunmugavelu M, Palanichamy S (1993) Transpiration through cuticle of the pholcid spider Crossopriza lyoni (Araneae: Pholcidae). J Ecotoxicol Environ Monit 3(2):151–154

Shunmugavelu M, Palanichamy S (1995) Feeding behavior of the tropical spider Crossopriza lyoni (Araneae: Pholcidae). Environ Ecol 13(2):375–377

Strickman D, Sithiprasasna R, Southard D (1997) Bionomics of the spider, Crossopriza lyoni (Araneae, Pholcidae), a predator of dung vectors in Thailand. J Arachnol 25(2):194–201. https://doi.org/10.2307/3705644

Turetzek N, Prpic NM (2016) Observations on germ band development in the cellar spider Pholcus phalangioides. Dev Genes Evol 226(6):413–422. https://doi.org/10.1007/s00427-016-0562-3

Vetter RS, Rust MK (2010) Periodicity of molting and resumption of post-molt feeding in the brown recluse spider Loxosceles reclusa (Araneae: Sicariidae). J Kans Entomol Soc 83(4):306–312. https://doi.org/10.2317/IKES0912.25.1

Vollrath F (1999) Biology of spider silk. Int J Biol Macromol 24(2–3):81–88. https://doi.org/10.1016/S1016-9353(99)80076-2

Wollf JO, Režač M, Krejčí T, Gorb SN (2017) Hunting with sticky tape: functional shift in silk glands of araneophagous ground spiders (Gnaphosidae). J Exp Biol 220(12):2250–2259. https://doi.org/10.1242/jeb.154682

WSC (2022) World Spider Catalog. Version 23.0. Natural History Museum Bern. http://wsc.nmb.ch. Accessed 21 January 2022

Yeng NGS, Mohtar JAB, Reddy GS, Srinivasulu K, Mahendran B, Reddy RS (2018) Isolation and purification of dragline silk protein from Crossopriza lyoni web. Web Acad J Biotechnol 7(2):041–048. https://doi.org/10.15413/jab.2018.0127

Zobel-Thropp PA, Mullins J, Kristensen C, Kronmiller BA, David CL, BreCI LA, Binford GJ (2019) Not so dangerous after all? Venom composition and potency of the Pholcid (daddy long-leg) spider Physocyclus globosus. Front Ecol Evol 7(256):1–16. https://doi.org/10.3389/fevo.2019.00256

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.