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Review of the *Eulimnadia* (Branchiopoda: Spinicaudata: Limnadiidae) of Tropical Asia, with the Description of a New Species

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We describe a new species of *Eulimnadia* from the Oriental region using museum specimens collected from India and fresh material from Thailand. This species has egg morphology resembling *E. magdalensis* s. lat. and *E. chaperi*, but distinctly differs from them by presence of a narrow depression at the polygon floor. We also comment on the species status of *E. khoratensis* from Thailand based on egg morphology, and present a taxonomic key for identification of tropical Asian species of *Eulimnadia*.

Key words: *Eulimnadia cryptus* sp. nov., *Eulimnadia michaeli*, Spiny clam shrimp, Diplostraca, Systematics, Synonymy.

BACKGROUND

The large branchiopods are a relatively small group of primitive crustaceans comprising the fairy shrimps (Anostraca), tadpole shrimps (Notostraca), and clam shrimps (Laevicaudata, Spinicaudata, Cyclestherida) (Brendonck et al. 2008). In Southeast Asia (SE Asia), clam shrimp and fairy shrimp occur mostly in seasonal, lentic, freshwater habitats (Rogers et al. 2013). Although tadpole shrimp have been found in India, China, and Japan, there are no records of this group in SE Asia (Rogers et al. 2013). The clam shrimps of SE Asia have only recently been examined (Rogers et al. 2016a b), most records (25 species) have been documented from the Indian subcontinent (Rogers and Padhye 2015; Padhye and Rabet 2017; Padhye and Kulkarni 2017; Padhye et al. 2018; Padhye and Lazo-Wasem 2018). During 2003–2010, there were only three records of clam shrimps from SE Asia; including *Eulimnadia* sp. from Northeast Thailand (Martin et al. 2003), *Cyclestheria hislopi* (Baird, 1859) from Cambodia, Malaysia, Singapore, Indonesia and Thailand (Martin et al. 2003), and *Eulimnadia* "*magdalensis*" Roessler, 1990 from Cambodia (Rabet 2010; Padhye and Rabet 2017). Following intensive collections of clam shrimp across Thailand since 2012, a total of eight species of clam shrimps (one species of *Cyclestheria*, two species of smooth clam shrimps, and five species of spiny clam shrimps) have so far been recorded in this country (Rogers et al. 2013 2016a b). Two species of smooth or laevicaudatan clam shrimps include the recently described; *Lynceus spinimanus* Rogers, Saengphan, Thaimuangphol and Sanoamuang, 2016, and *Lynceus planifascius* Rogers, Saengphan, Thaimuangphol and Sanoamuang, 2016 (Rogers et al. 2016a). In addition, five species of spiny or Spinicaudatan clam shrimps consist of (1) *Cyzicus pilosus* Rogers, Thaimuangphol, Saengphan and Sanoamuang, 2013; (2) *Leptestheria*

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serracauda Rogers, Dadseepai and Sanoamuang, 2016; (3) *Eulimnadia khoratensis* Rogers, Dadseepai and Sanoamuang, 2016; (4) *Eulimnadia indocylindrova* Durga Prasad and Simhachalam, 2004; and (5) *Eulimnadia compressa* (Baird, 1860) (Rogers et al. 2013 2016b). However, our re-examination of specimens of the species previously identified as *E. compressa* demonstrates it to be a new species. Thus, we describe *Eulimnadia cryptus* sp. nov., correct a taxonomic error, and present keys to the Asian *Eulimnadia* species (note Timms and Rogers (2020) provide diagnostic features of *Eulimnmadia* and *Paralimnadia*) based on egg morphology.

MATERIALS AND METHODS

Specimens were examined using a Wild M8 and a Zeiss Stemi 2000 stereomicroscope outfitted with a Q-Imaging Micropublisher 5.0 RTV digital camera utilizing Q-Capture 7.0 acquisition software. Scanning Electron Microscopy (SEM) images of the eggs were taken with a Philips XL-30 Environmental Scanning Electron Microscope at an accelerating voltage of 10 kV using the chemical drying protocol given by Nation (1983).

RESULTS

SYSTEMATICS

Limnadiidae Baird, 1849

Eulimnadia cryptus sp. nov.

(Figs. 1, 2)

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= *Eulimnadia* sp. Rogers et al. 2013a: 76, Fig. 1D

= *Eulimnadia compressa* (Baird, 1860) in Rogers and Padhye 2015: 401; Rogers et al. 2016b: 567, Fig. 1A–C

Distribution: India, Thailand, and Cambodia (Rabet 2010; Rogers et al. 2013a; Rogers and Padhye 2015; Padhye and Rabet 2017; this study).

Habitat: All our collections were from anthropogenic rainwater puddles, table drains, rice paddies, and storm water retention basins.

Material examined: India, Tamil Nadu: Chingleput, unused rice patty six kilometres north of Mahabalipuram, 12.67°N, 80.17°E, 24 October 1980, D. Belk, USNM 1143259, 43 hermaphrodites (see comments below under "Types"). Thailand, Chon Buri Province: Highway 3 (Sukhumvit Road), between Sattahip and Nang Sar-re, pool formed in road ruts on east side, 12°43'24.75"N, 100°53'17.52"E, 12 June 2015, D.C. Rogers, L. Sanoamuang, N. Sanoamuang coll, 23 hermaphrodites. Khon Kaen Province, Nai Muang District, table drain on Jenjobtid Road, 16°05'42.57"N, 102°44'37.73"E, 9 May 2015, P. Dadseepai coll, 46 hermaphrodites. Rice paddies on south side of Highway 208, 16°19'18.09"N, 102°51'35.63"E, 19 June 2015; D.C. Rogers, and P. Dadseepai coll., 64 hermaphrodites. Muang District, Ban Pet, Bypass Road, 16°25'50.71"N, 102°46'00.23"E, 12 June 2015, D.C. Rogers, and P. Dadseepai coll., 136 hermaphrodites. Muang District, Ban Pet, Bypass Road, 16°25'59.61"N, 102°45'55.98"E, 12 June 2015, D.C. Rogers and P. Dadseepai coll., 71 hermaphrodites. Muang District, Ban Pet, Bypass Road, 16°26'50.86"N, 102°46'08.23"E, 12 June 2015, D.C. Rogers and P. Dadseepai coll., 13 hermaphrodites. Ban Phai District, Ban Nong Wang Rai, 16°05'42.57"N, 102°44'37.73"E, 9 June 2015, P. Dadseepai coll., 44 hermaphrodites. Maha Sarakham Province: Agricultural drain on west side of 2300 Road, 16°14'14.51"N, 102°54'52.19"E, 19 June 2015, D.C. Rogers and P. Dadseepai coll., 8 hermaphrodites. Roi Et Province: Roadside rice paddy, across road from Lao Luang Sub District Administration Organisation Public Park on west side of Highway 214, between Kaset Wisai and Si Khot, just east of Lao Luang, 15°43'33.51"N, 103°34'45.34"E, 23 May 2015, P. Dadseepai coll., 62 hermaphrodites. Udon Thani Province: Rice paddy on north side of Highway 2023 east of Phan Don, 17°07'27.48"N, 102°58'25.22"E, 20 June 2015, D.C. Rogers, S. Tiang-nga, and S. Mali-ong coll., 58 hermaphrodites. Rice paddy on north side of Highway 2023, between Kumphawapi and Wiang Kham, 17°06'39.31"N, 103°02'18.81"E, 20 June 2015, D.C. Rogers, S. Tiang-nga, and S. Mali-ong coll., 107 hermaphrodites. Rice paddy 1.7 km east of Highway 2023, between Tat Thong and Champi, 17°01'22.90"N, 103°08'32.22"E, 20 June 2015, D.C. Rogers, S. Tiang-nga, and S. Mali-ong coll., 4 hermaphrodites. Rice paddy on west side of Highway 2 n 023, just north of Champi, 16°59'25.35"N, 103°09'08.90"E, 20 June 2015, D.C. Rogers, S. Tiang-nga, and S. Mali-ong coll., 107 hermaphrodites.

Types: We designate material from USNM 1143259 as the types. The holotype is deposited at the United States Natural History Museum (Smithsonian), under collection number USNM 1604207. The remaining *E. cryptus* sp. nov. are designated as paratypes and are retained as USNM 1143259. The original lot also contained nine specimens of *Eulimnadia indocylindrova* Durga Prasad and Simhachalam, 2004 (fide Padhye et al. 2015). The

material of *E. indocylindrova* is now USNM 1604210.

Diagnosis: (Modified from Rogers et al. 2016b). Defining characters only visible with scanning electron microscopy. Mature egg (Fig. 1A–C) with 14–18 polygons, each with deep, sloping sides, towards a shallowly furrowed base, often with several small pits (Fig. 1D). Polygons margined with narrow ridges, bearing uneven edges, which can project as flat, narrow lobes especially at ridgeline intersections. Each depression with a central, transverse wrinkled groove, lacking subtending pores. Eggs 120–130 μm in diameter.

Description: Egg as for diagnosis above. Description based on putative hermaphrodite. Ocular tubercle prominent (Fig. 2C, D), overreaching rostrum. Head broadly rectangular, broader than ocular tubercle. Contiguous compound eyes large, subspherical, ~0.85x ocular tubercle width. Naupliar ocellus subtriangular, lying just posterior to, or slightly dorsal and posterior to rostrum. Ocular angle abruptly angulate to rostrum. Rostrum pronounced, broadly rounded to truncated or acute, slightly upturned apically, 0.7x ocular tubercle width. Angle between rostrum and frons 100° to 90°. Rostrum even with head ventral surface. Pedunculate frontal organ length approximately 0.60x to 1.2x distance of organ from ocular tubercle. Frontal organ prominent, pedunculate, directed anteriorly, and elongate ovate to subtriangular. First antennae well below and posterior to rostrum, pedunculate, and 0.5x as long as second antennal peduncle. Second antennae 2 to 2.5x head length. Second antennal peduncle subcylindrical, subequal in length to head, slightly geniculate, and bearing dorsal transverse rows of spiniform setae. Second antennal anterior flagellum (exopod) with six or seven annulations, each dorsally with a transverse row of setae. Posterior flagellum (endopod) with eight or nine such annulations, and subequal in length to anterior flagellum. Both flagellae with a ventral, longitudinal row of long plumose natatory setae, about 0.7x peduncle length.

Carapace (Fig. 2A, B) broadly oval, with three or four well separated, shallowly impressed, obscure

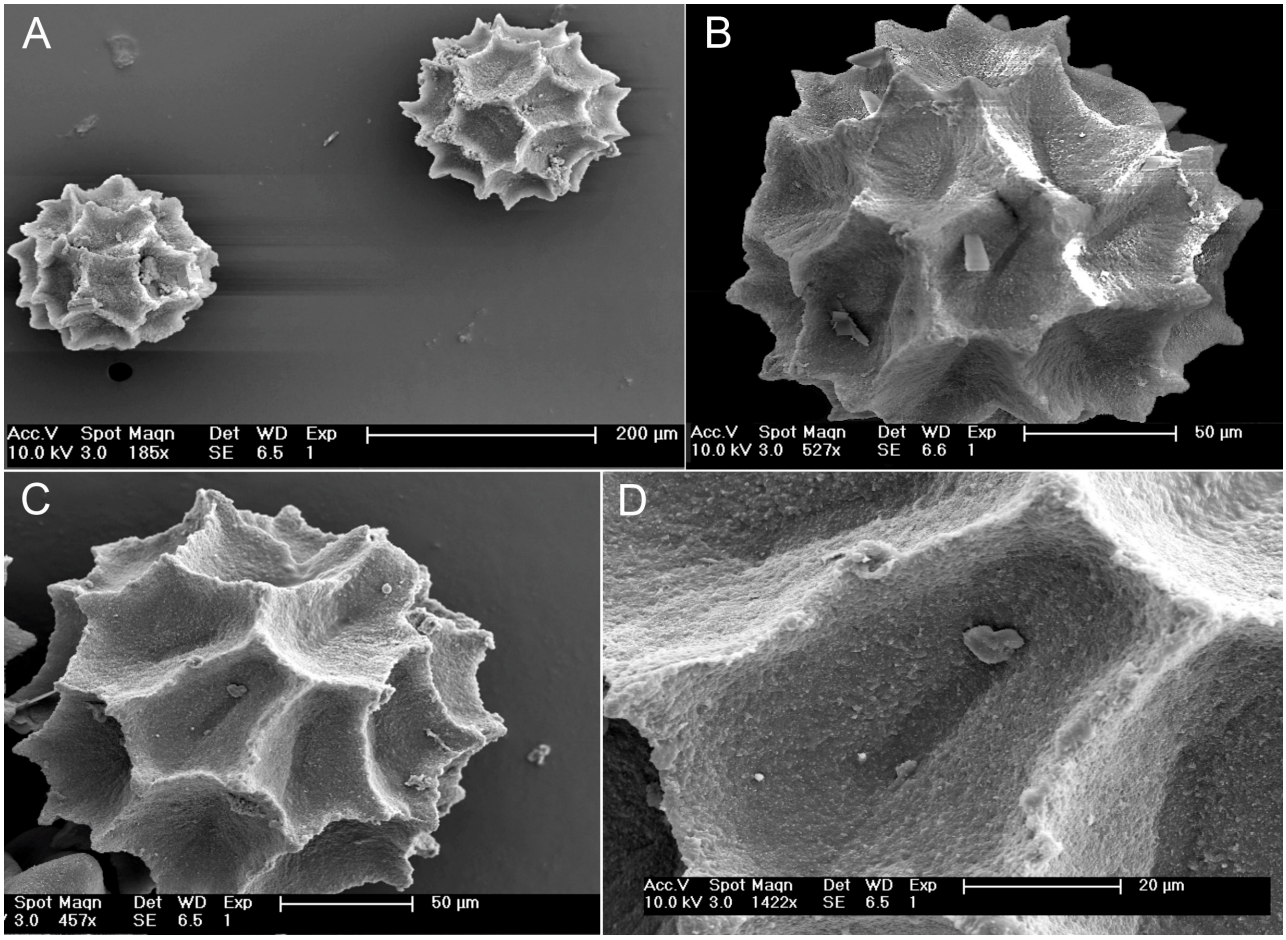

Fig. 1. *Eulimnadia cryptus* sp. nov. A–C, Resting eggs overall structure and variation; D, Single egg polygon. Scale bars: A = 0.2 mm; B and C = 0.05 mm; D = 0.02 mm.

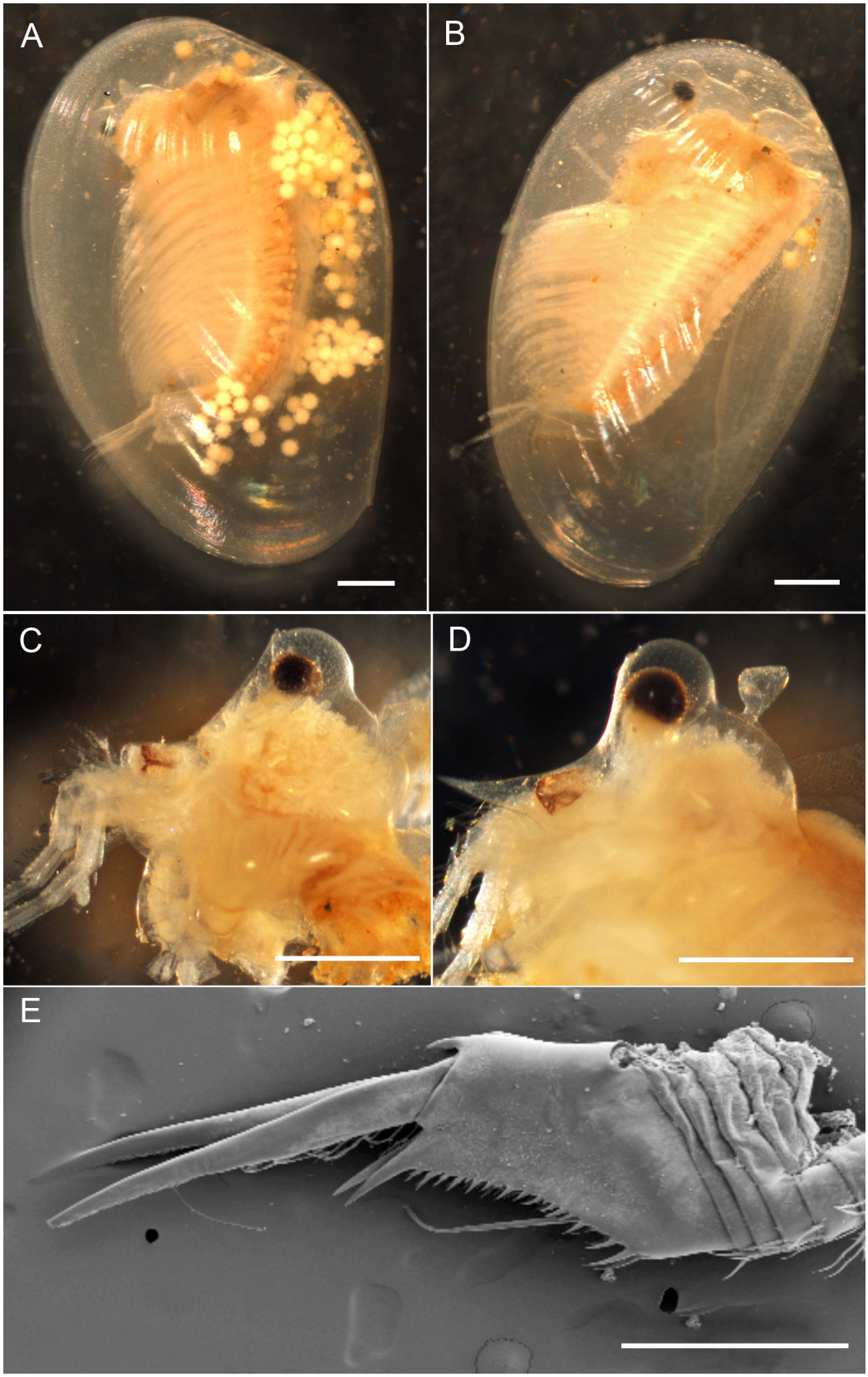

Fig. 2. *Eulimnadia cryptus* sp. nov. A, Hermaphrodite habitus; B, Male habitus; C, Hermaphrodite head; D, Male head; E, Hermaphrodite telson. Scale bars: A–E = 0.5 mm).

growth lines. Carapace surface between growth lines smooth. Umbone absent. Adductor muscle scar broad, oblong, length ~2x width. Fourteen to sixteen pairs of thoracopods, with tenth and eleventh pairs bearing dorsally elongated flabellae for carrying eggs. Body segments posterior to eleventh thoracopod pair dorsally with transverse row of plumose setae, diminishing in length serially in posterior abdominal segments.

Telson (Fig. 2E) with thirteen to fifteen pairs of posterior spines borne on the posteriolateral ridges. Anterior most spine pair directed dorsally or subdorsally, often larger than remaining pairs. Spines spaced unevenly, sometimes separated by more than spine basal width. Spines uneven in size, with smaller spines having a basal width ~0.7x the larger spines basal width. Caudal filaments originating on mound on telson posterior surface between the ridges at or about the fourth pair of spines. Telson posteriolateral ridges each terminating in an elongated spiniform projection, 3.5 to 4.0x nearest spine length. Cercopods projecting posteriorly from the ventral surface of the telson, each subtended by an anteriobasal spiniform projection, directed posteriolaterally over the base of the cercus. Cercopod length ~1.2x telson length, margined dorsomedially with a longitudinal row of long plumose setae, extending from base distally to ~75% point. Setal row terminating in a short spine.

Differential diagnosis: Adult morphology not readily, nor reliably distinguishable from other Asian *Eulimnadia* species. The specific diagnostic characters are limited to the eggs in this genus, and are only visible with scanning electron microscopy. *Eulimandia cryptus* sp. nov. is separated from all other *Eulimnadia* species except *E. chaperi* and *E. magdalensis* by the form of the mature egg which is subspherical and bears numerous broadly rounded polygons, each with sloping sides, and a shallowly furrowed base. The polygons are margined with narrow ridges, bearing uneven, crenulated edges, which may project as flat, narrow lobes especially at ridgeline intersections. This basic egg morphology is shared with *E. chaperi* from India, and *E. magdalensis* from the Americas and possibly Cambodia. *Eulimnadia magdalensis* has the depressional floors smooth, whereas they are ornamented in *E. cryptus* sp. nov. and *E. chaperi*. Both *E. cryptus* sp. nov. and *E. chaperi* have a transverse groove in the centre of each depression. However, in *E. chaperi* the groove is subtended by four pores (two on each side), with connecting slits to the central groove.

Comments: *Eulimnadia cryptus* sp. nov. was previously confused with *E. chaperi* and *E. compressa* by Rogers et al. (2016b). Baird (1860) described *Eulimnadia compressa* from India, but the types were lost and the description was limited to the carapace. Simon (1886) described *E. chaperi* from India based on specimens at the Paris Museum of Natural History, but Daday (1926) treated it as a junior synonym of *E. compressa*. Rabet (2010) pointed out that the egg morphology of *E. compressa* was unknown and the five separate lots identified by Daday as *E. compressa* did not all have the same egg morphology (where eggs were present), although they were grossly compatible with *E chaperi* and the New World species *E. magdalensis* Roessler, 1990. Using SEM, Rabet (2010) demonstrated that the egg depressions differ significantly in the ornamentation of the depression bottoms (compare Rabet's (2010) fig. 2H and fig. 3F, and the images in Padhye and Rabet's (2017) fig. 6) between the taxa. Rabet (2010) reported material from Cambodia that resembled *E. magdalensis*. The eggs of *E. magdalensis* are similar to *E. chaperi*, but with less uniform polygons and more elongated crests (see Pereira and Garcia 2001). Thus, Rogers et al. (2016b) stated it was likely that Rabet's Cambodian material, as well as similar material they collected throughout Thailand belonged to *E. compressa* (in error; they had meant *E. chaperi*). Rabet (2010) and Padhye and Rabet (2017) redescribed *E. chaperi* using the type material at the MNHN, and resolved the confusion surrounding *E. compressa* and *E. chaperi*, demonstrating that *E. compressa* is a nomen dubium, and that *E. cryptus* sp. nov. was an undescribed taxon.

Eulimnadia michaeli Nayar and Nair, 1968

= *Limnadia michaeli* (Nayar and Nair, 1968)
= *Eulimnadia khoratensis* Rogers, Dadseepai, and Sanoamuang, 2016

New Status

Comments: This species was treated as a species inquirenda by Rogers and Padhye (2015), based on the lack of type material and poor descriptions. In 2016, Rogers et al. (2016b) described what was believed to be a new species from the Khorat Plateau in north central Thailand. Padhye and Kulkarni (2017) reported *E. khoratensis* from India and examined the extensive morphological variation in this species. All these authors were unaware of the paper by Samyiah et al. (1985), wherein scanning electron micrograph images of topotype material of *E. michaeli*, including the egg, were presented. There is little doubt that, based on the images of the eggs, *E. khoratensis* is a junior synonym of *E. michaeli*. Rogers et al. (2016: 570) wrote: "… it is possible that *E. khoratensis* n. sp. could be a junior synonym of *E. gunturensis* Radhakrishna and Durga Prasad, 1976, *Eulimnadia michaeli* Nayar

and Nair, 1968, or *Eulimnadia ovata* Nayar, 1965 if the morphologies of the eggs of these species are ever properly described according to modern standards."

This species ranges across tropical Asia from India, Sri Lanka, and Thailand (Rogers and Padhye 2015; Rogers et al. 2016a; Padhye and Kulkarni 2017). The types of *E. michaeli* are presumed lost, but the types of *E. khoratensis* are deposited at the Muséum national d'Histoire naturelle, Paris (MNHN-IU-2014-17472).

Keys to the Tropical Asian *Eulimnadia* (key adapted from Padhye et al. 2018)

1 Egg cylindrical 2
- Egg spherical/ sub-spherical 3
2(1) Flat ends dilated into flanges ..... *Eulimnadia bondi*
- Domed ends without flanges ..... *Eulimnadia braueriana/ Eulimnadia indocylindrova* (Note: possible synonyms)
3(1) Polygons separated by thin, narrow ridges 4
- Polygons separated by thick, rounded ridges; polygons with a narrow slit in the central depression; egg with 18–26 polygons ..... *Eulimnadia michaeli*
4(3) Polygons with floors bearing obvious central pores or grooves 5
- Polygon depression floors smooth, unornamented; egg with 20–26 polygons ..... *Eulimnadia magdalensis*
5(4) Egg with 12–16 polygons; depressions ornamented randomly with small groove/s and 1–3 pores on either side of the groove ..... *Eulimnadia chaperi*
- Egg with 14–18 polygons; depressions ornamented with a narrow central groove ..... *Eulimnadia cryptus* sp. nov.

CONCLUSIONS

A description of a new species of Spinicaudatan clam shrimp, *Eulimnadia cryptus* sp. nov., from India and Thailand, is presented. Morphologically, it is closely related to the American *E. magdalensis* and the Indian *E. chaperi*, but differs distinctly from other congeners by the unique form of the mature egg. In addition, keys to the Tropical Asian *Eulimnadia* adapted from Padhye et al. (2018) are presented.

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Authors' contributions: SP made the initial observations concerning the new species. DCR made subsequent observations, identified the synonymies and wrote the manuscript. LS prepared the Background. All authors discussed the results and contributed to the final manuscript.

Competing interests: The authors declare that they have no competing of interests.

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Consent for publication: Not applicable.

Ethics approval consent to participate: Not applicable.

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