

**Molecular and morphological confirmation of an invasive American isopod;  
*Livoneca redmanii* Leach, 1818, from the Mediterranean region to Lake Qaroun,  
Egypt**

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## ABSTRACT

In 2015, unknown cymothoid isopod parasites were found in Lake Qaroun, a high-salinity closed inland lake in north-central Egypt, which were linked to substantial fish mortalities. The parasites infected a wide range of commercially important fish hosts, including Redbelly tilapia, *Tilapia zillii*, European Seabass, *Dicentrarchus labrax*, Common Sole, *Solea solea*, and mullets *Mugil* spp. and *Liza* spp., and continue to present a threat to local fisheries. A number of recent publications (in aggregate) have suggested this introduction reflected a near simultaneous introduction of up to four cymothoid species in Lake Qaroun; however, many of the provided photographs and illustrations were incorrect at the generic and species level. Cymothoid isopod samples were collected from Shakshouk village on Lake Qaroun. An integrative taxonomic approach was applied in the current study, incorporating traditional taxonomic analysis and DNA barcoding through mitochondrial *COI* gene sequencing, as well as genetic phylogenies to delimit isopods collected in Lake Qaroun. All isopods examined were identified as *Livoneca redmanii* Leach, 1818, an estuarine-marine species native to the western Atlantic. These samples were genetically and phylogenetically identical to their counterparts collected from Chesapeake Bay in the East of the USA. This species was characterized by pigmentation on cephalon, pereonites, pleonites, telson, pereopods and slightly bent to the left. A review of the recent literature of cymothoids in Lake Qaroun and adjacent marine areas concluded that the majority of individuals identified as species other than *L. redmanii* were actually *L. redmanii*, or unidentifiable juvenile cymothoids, with the exception of a juvenile *Anilocra* sp.. Likewise, *L. redmanii* is also present in eastern Mediterranean and Suez Canal. The likely introduced vector into Lake Qaroun are fish stockings originating from the Mediterranean Sea and River Nile estuary (as is surmised with the introduced American ctenophore species, *Mnemiopsis leidyi*). This information can help fishery managers better understand parasite-host dynamics in Lake Qaroun and develop biocontrol strategies to mitigate its impact in Lake Qaroun and limit its spread among adjacent lacustrine, estuarine and coastal systems.

## INTRODUCTION

Invasive species pose a significant threat to global biodiversity and can disrupt ecosystem services and impact human health, and well-being (Pejchar and Mooney, 2009; Katsanevakis *et al.*, 2014 and Early *et al.*, 2016). In particular, aquatic invasive species have the potential to severely harm commercial, recreational, and subsistence

fisheries, thereby disrupting local economies and communities that are dependent on these fishery resources (Bax *et al.*, 2003).

In 2015, unknown cymothoid parasites appeared in Lake Qaroun, a high-salinity inland lake in north-central Egypt, which has the highest fisheries yield of any Egyptian inland lake and constitutes a large portion of the local economy by supplying fish and shellfish to regional markets throughout Egypt (Hassan *et al.*, 2014). Moreover, the lake supports large populations of migratory and resident bird species, which are the foundation of a burgeoning ecotourism industry (Wafik *et al.*, 2011). A number of recent studies have suggested that there has been a near simultaneous introduction of four species of cymothoid isopod in Lake Qaroun namely: *Anilocra physodes* Linnaeus, 1758 and *Nerocila orbignyi* Guérin-Méneville, 1832, both widely reported in the Mediterranean (Bruce, 1987b; Öktener, *et al.*, 2018); *Renocila thresherorum* (Williams and Bunkley-Williams, 1980), endemic to the eastern Pacific (Williams and Bunkley-Williams, 1980), but reported from the Suez Canal (Youssef *et al.*, 2014); and *Livoneca redmanii* Leach, 1818, native to the western Atlantic and Caribbean (Bruce, 1990), but also recently reported from coastal Egypt (Abdel-Latif *et al.*, 2016; Mahmoud *et al.*, 2016, 2017, 2019; Younes *et al.*, 2016; Shaheen *et al.*, 2017; Ali and Aboyadak, 2018; Helal and Yousef, 2018). Additionally, other recent publications have reported a number of non-native cymothoids in the eastern Mediterranean/Suez Canal, including *R. thresherorum*, *Cymothoa exigua* Schioedte and Meinert, 1884 (known from Gulf of California to Ecuador (Williams and Bunkley-Williams, 2003), but recorded in the Red Sea (Al-Zubaidy and Mhaisen, 2013) and *Anilocra meridionalis* Richardson, 1914 (known from the Galapagos and central North Pacific (Brusca, 1981) in the Suez Canal (Youssef *et al.*, 2014) and *Anilocra leptosoma* Bleeker, 1857 (known from southern India to Australia (Bruce, 1987a; Aneesh *et al.*, 2019) in coastal Egypt (Rania and Rehab, 2015). However, much of the illustrated data conflicted with taxonomic assignments at the generic level, casting doubt on these identifications, as it will be discussed later herein.

The parasites infected several commercially important fish species, including Redbelly Tilapia, *Tilapia zillii*, European Seabass, *Dicentrarchus labrax*, soles, *Solea* spp. and mullets *Mugil* spp. and *Liza* spp., and continue to present a threat to local fisheries. Since this introduction, numerous mixed-species fish kills containing hundreds of individuals have occurred and nearly all mortalities were infected with cymothoid parasites (Younes *et al.*, 2016; Mahmoud *et al.*, 2017; Mohammad-Geba, personal observation). Recent work has indicated that these mortalities may have resulted from the synergistic effects of parasitic infection (which can cause gill/tissue damage, DNA damage (Mahmoud *et al.*, 2016; Elgendy *et al.*, 2017; Ali and Aboyadak, 2018; and Helal and Yousef, 2018), elevated pollutants (e.g., iron, cadmium, lead, ammonia, etc. (Abdel-Latif, 2016; Mahmoud *et al.*, 2016; Elgendy, *et al.*, 2017), and bacterial infections (e.g., *Vibrio* spp. and *Aeromonas* spp. (Younes *et al.*, 2016; Elgendy *et al.*, 2017). At the same time, annual fishery yields in Lake Qaroun dropped from a record high of 4,518 t in 2014 to 1,124 t in 2015, falling even further to 873 t in 2016 (GAFRD, 2017). These marked declines in catches and physiological impacts have raised serious concerns about the health of fishery resources in Lake Qaroun. Furthermore, uncertainty surrounding the taxonomic identity of the cymothoids has hampered the development of biocontrol strategies as well as a full understanding of invasion and host-parasite dynamics in Lake Qaroun.

Cymothoids are obligate fish parasites that typically parasitize the skin, gills, buccal cavities and abdominal cavities of their hosts (Thatcher, 2000; Smit *et al.*,

2014; Hata *et al.*, 2017). After hatching, free-swimming larval isopods (mancae) seek fish hosts using egg yolk stores for up to 1-2 weeks (Sandifer and Kerby, 1983). After finding an appropriate host, the manca will molt into a functionally male juvenile form. All cymothoids are protandrous hermaphrodites, with the juvenile-male-to-adult-female transformation dependent upon a variety of factors, including the presence of adult females on the host fish, which will inhibit male-to-female transformation (Lincoln, 1971). In natural systems, the effects of cymothoid infections are generally thought to be minor (particularly at the population level), but can cause localized tissue damage, reductions in reproductive potential and fitness, behavioral alterations and even death in extreme cases (Fogelman *et al.*, 2009; Smit *et al.*, 2014). High fish stocking densities can exacerbate these effects and alter fish host preferences, particularly when parasites are exposed novel/introduced habitats or novel host species (Horton and Okamura, 2003; Smit *et al.*, 2014).

The Cymothoidae was one of the first isopod families to be described and many of the original descriptions for species described in the 1800s are too brief to properly delimit species, and in some instances the type material may be lost. In recent decades, a large number of species have been redescribed and questions surrounding generic classifications have mostly been resolved, (Trilles, 1976; Bruce, 1986, 1987a,b,c, 1990; Smith *et al.*, 2014; van der Wal *et al.*, 2017), but uncertainties surrounding the taxonomic status of a number of important species remain. One major uncertainty pertains to the validity and identity of *Livoneca ovalis* Say 1818 and its relationship to *L. redmanii*. Specifically, whether *L. redmanii* is: 1) a single species distributed from Massachusetts, along the eastern US through the Caribbean to South America or 2) a species complex comprising *L. ovalis* distributed along the eastern US and possibly along the Central American coast, and *L. redmanii* distributed in the Caribbean and along eastern South America (Bruce, 1990; Williams and Bunkley-Williams, 1996), with possible distributional overlap. In a major revision of several cymothoid genera, Bruce (1990) reported that *Livoneca* is a New World genus containing only two accepted species: *L. redmanii* (including *L. ovalis* as a junior synonym, which was also upheld implicitly by Trilles (1994), endemic to the western Atlantic and *Livoneca bowmani* Brusca 1981, native to the eastern Pacific. However, differences in host specificity (Williams and Bunkley-Williams, 1996), male-female pair formation (Williams and Bunkley-Williams, 1996) and morphology of the pleotelson and uropods (Niel L. Bruce, Queensland Museum, personal communication) suggest *L. ovalis* may be a valid species. Say (1818) did not list a type locality in the original description of *L. ovalis* (though it's likely in the vicinity of Philadelphia, Pennsylvania, USA) and the type material is now lost (Paul Calloman, The Academy of Natural Sciences of Drexel University, personal communication), further confounding these taxonomic uncertainties.

Genetic techniques, such as DNA barcoding, offer new tools for ecological investigations, including identifying unknown samples, investigating cryptic diversity/invasive species and examining phylogenetic diversity (Hebert *et al.*, 2003; Kress *et al.*, 2015). It can be extremely powerful in examining species relationships when employed within an integrative taxonomic framework (i.e., also incorporating morphological, ecological and other data sources; Padial *et al.*, 2010; Schlick-Steiner *et al.*, 2010; Zhang *et al.*, 2018). However, these analyses can be confounded by uncertainty regarding the taxonomic identification and provenance of genetic sequences contained in public databases. Confidence in determining similarities among sequences is greatly increased when there are corresponding voucher specimens, photographs, and metadata associated with reference sequences. In 2011,

the Chesapeake Bay Barcode Initiative (CBBI) began creating complete *COI-5P* barcode libraries for fish and macroinvertebrates of Chesapeake Bay and surrounding mid-Atlantic, which includes photographic, tissue and specimen vouchers that were deposited into the Smithsonian National Museum of Natural History collections (e.g., Aguilar *et al.*, 2017). Cymothoid sequences generated by the CBBI were incorporated into the present study to assess relationships among individuals collected in Lake Qaroun and other isopods with high confidence.

The present study utilized an integrative taxonomic approach incorporating DNA barcoding and traditional morphological analysis to accurately identify invasive isopods collected in Lake Qaroun. Additionally, the study comments on biocontrol strategies to mitigate the isopod's impact on fishery resources in the lake and limit its spread within the region.

## MATERIALS AND METHODS

### Study area

Lake Qaroun (also known as Birket Qarun or Lake Karoun) is a closed high-salinity lake occupying roughly 230 km<sup>2</sup> and located approximately 90 km south of Cairo, Egypt (Figure 1). It was once freshwater (known in antiquity as Lake Moeris), much larger and directly connected to the Nile River via the Bahr Yusuf canal (Baoumy *et al.*, 2010). Over time the functionality of the Bahr Yusuf canal decreased, causing the lake to shrink in volume and steadily increase in salinity, which extirpated the majority of freshwater fauna and decimated commercial and subsistence fisheries (Ishak, 1980).



**Fig. 1:** Map of Lake Qaroun region in north-central Egypt

Currently, Lake Qaroun is hypersaline, fluctuating from 40-45 psu with freshwater inputs derived entirely from agriculture drainage sourced from the Nile River. Beginning in 1928, the lake has been stocked with several marine and estuarine fishes, notably mullets, *Mugil* spp. and *Liza* spp., Gilthead Sea Bream, *Sparus aurata*, European Seabass, Common Sole, and shrimps *Metapenaeus* spp. and *Penaeus* spp. (Ishak, 1980; El-Serafy *et al.*, 2014). A few species naturally spawn in the lake (e.g., *T. zillii*, Jumping Mullet *Liza saliens*, and *Solea* spp.), but many are collected from northeastern Egyptian estuaries and coastal lagoons and stocked by the Egyptian

General Authority for Fish Resources Development (GAFRD) semi-annually, particularly mullet fry (El-Serafy *et al.*, 2014). Additional non-target species, such as African pupfishes *Alphanius* spp., eel *Anguilla* sp., Big-scale Sand Smelt *Atherina boyeri* and Double-ended Pipefish *Trachyrhamphus bicoarctatus* were accidentally introduced via stocks of mullet and other fish fry (Ishak, 1980; Khalaf Allah, 2014). These stockings are the assumed introduction vector for the American ctenophore species *Mnemiopsis leidyi* (El-Shabrawy and Dumont, 2016) and mosquitofish *Gambusia affinis/holbrooki* (Khalaf Allah, 2014), which were previously reported from the Mediterranean. Conversely, the cosmopolitan brine shrimp *Artemia franciscana* was likely introduced into Lake Qaroun via the transportation of cysts on the bodies of migratory birds (Sheir *et al.*, 2018).

#### Parasite collection and morphological examination

In July 2015, fifteen Redbelly Tilapia were collected from Lake Qaroun near Shakshouk Village (coordinates: 29.465229 N, 30.707076 E) by local fishermen. Ten tilapia (66%) were infected with unknown cymothoid isopod parasites. Two fish were infected with at least a male and a female parasite (Figure 2), present in both the gill chamber and buccal cavity. Five fish possessed multiple males and the remaining three fish possessed a single male located in the gill chamber. Ten isopods were randomly selected and a 50 mg piece of abdominal musculature was dissected from each individual for DNA analysis.



**Fig. 2:** *L. redmanii* female: (a) dorsal view; (b) ventral view; and male: (c) dorsal view; (d) ventral view. Isopods were collected from Lake Qaroun, Egypt. Scale bar; 1 mm.

The remaining isopods (10 males and 1 female) were preserved in 70% ethanol and morphologically examined in detail according to Brusca (1981), Bruce (1990), and Bakenhaster (2004) and photographed with a digital camera attached to a stereo microscope, and prepared for line drawing. The present study recorded the following measurements (biometrics): total body length, body width, cephalon length, compound eye length and width, antenna and antennule length, telson and uropod length, and eye/cephalon length ratio, using a calibrated ocular micrometer under a

stereo microscope to the nearest millimeter (mm). Lastly, the antennae, antennule, pereopods, pleopods, and uropods were dissected from a subset of isopods and then cleared in phenol and examined according to Thatcher *et al.* (2007).

### Genetic Identification

#### DNA extraction

After dissection, tissue samples were placed in a 1.5 mL Eppendorf tube containing 5 % Chelex<sup>®</sup> 100 sodium form resin (Sigma-Aldrich, Madrid, Spain) in TE buffer (pH 8) following Wolff and Gemmel (2008). Then 2.4 U of Proteinase K (ThermoFisher) was added to each tube. Samples were incubated at 55 °C with vortexing at 30 min intervals for 5 hours. Samples were then boiled in a 100 °C dry bath for 20 minutes and then stored at 4 °C until DNA amplification.

#### PCR amplification of COI gene

The target region of the *COI* gene was amplified by PCR using the cycling conditions and universal primer pairs, jgLCOI490 and jgHCO2198, as previously described by Geller *et al.* (2013). The amplification reactions were performed in a total volume of 50  $\mu$ L, of which 3  $\mu$ L of total genomic DNA, 0.4  $\mu$ M of each primer, and 200 ng  $\mu$ L<sup>-1</sup> of bovine serum albumin (BSA) were used. The PCR was performed by using 2x DreamTaq<sup>™</sup> Green PCR Master Mix (Cat. no. K1081, ThermoFisher) according to the manufacturer's instructions. The PCR products (3  $\mu$ L) were electrophoresed in a 2% (w/v) agarose gel, containing 0.5  $\mu$ g mL<sup>-1</sup> of ethidium bromide, and visualized under UV light. The PCR products (~650 base pairs) were sent to MACROGEN Inc. (Seoul, South Korea) for standard Sanger sequencing.

#### Sequence analyses

Mitochondrial *COI* sequences were reviewed and manually trimmed whenever necessary. Edited sequences were compared to archived reference sequences in GenBank using BLAST (Altschul *et al.*, 1990). Isopod sequences were aligned using CLUSTALW, integrated with the program MEGA 6 (Tamura *et al.*, 2013) and haplotypes were determined using DNasp6 (Rozas *et al.*, 2017). Twenty-one publicly available *COI* cymothoid sequences were downloaded from GenBank and BOLD, including *Anilocra brillae*, *A. chromis*, *A. haemuli*, *A. physodes*, *Ceratothoa collaris*, *C. italica*, *C. verrucosa*, *Cterissa sakaii*, *Cymothoa eremita*, *C. excisa*, *C. pulchrum*, *Elthusa sacciger*, *E. vulgaris*, *Ichthyoxenus tanganyikae*, *Joryma hilsae*, *Livoneca redmanii*, *Mothocya parvostis*, *M. collettei*, *Nerocila longispina*, *N. phaiopleura*, and *N. japonica*. The primitive free-living isopod *Phreatomerus latipes* was included as an outgroup. All downloaded sequences were aligned with the resulting Lake Qaroun haplotypes using CLUSTALW and the best substitution models were selected with the ModelTest algorithm, both integrated in MEGA 6 software (Tamura *et al.*, 2013). Bayesian inference (BI) was carried out using MrBayes 3.2.1 (Ronquist *et al.*, 2012). Four Markov Chains Monte Carlo (MCMC) chains were analyzed for 10 million (ngen=10,000,000) generations, saving a tree each 1,000 generations. The subsequent analysis started when the average standard deviation of split frequencies reached 0.001. Tracer 1.7 (Rambaut *et al.*, 2018) was used to calculate effective samples size and number of burn-ins, which determined that 25 % of the saved trees are to be discarded as burn-ins. This information was transferred to MrBayes 3.2.1. to construct the summarized tree, which was later annotated through *TreeAnnotator v1.6.1 software* (Rambaut and Drummond, 2010), and finally visualized using Fig Tree v 1.3.1.

To confirm the identifications of cymothoid isopods reported in Lake Qaroun and adjacent coastal areas, in consultation with Niel Bruce (Queensland Museum, Australia), we examined the photographs and illustrations provided in all the recent

publications documenting cymothoids in Lake Qaroun, as well as additional publications from the Suez Canal and Egyptian Mediterranean coast.

## RESULTS

### *L. redmanii* – female and males collected from Lake Qaroun

*L. redmanii* female and males closely match description and line drawings of *L. redmanii* holotype (type locality: Jamaica) and a *L. redmanii* collected from Colombia (USNM:IZ: 171630) presented in Bruce (female only, 1990) and American samples collected by the CBBI (UNSM:IZ: 1286837-126838, 1286855-1286857, 1409696-1409699), *Levonica* sp. by Brusca (1981), early stage *L. redmanii* manca from the Gulf of Mexico (Bakenhaster, 2004), and juvenile from Florida (Poulakis *et al.*, 2010).

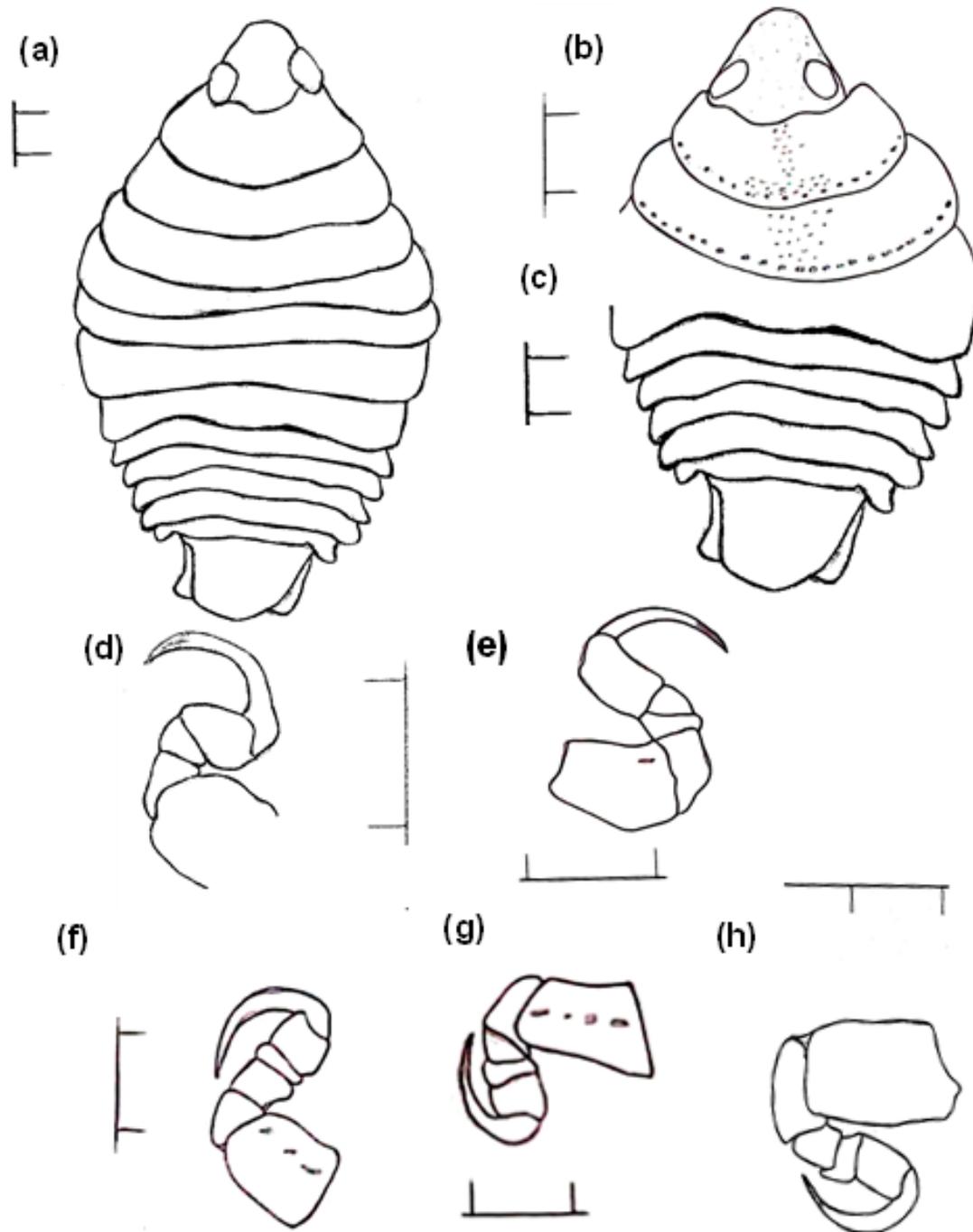
Body smooth, weakly vaulted, and twisted sinisterly. Color in life creamy white-yellow, (creamier in ethanol) with stippled pigmentation, not arranged in distinct longitudinal bands. Chromatophores formed thin stripes along posterior margins of cephalon, pereon, and pleon segments, excluding pleotelson. Cephalon anterior margin folded back, concave, pointed, with prominent raised rostral process. Pereon had seven segments, pleon with five segments, excluding pleotelson. Seven pereon segments thoroughly pigmented with dense clusters of chromatophores near posterior margin, but posterior margin unpigmented. Coxal plates as long as or longer than respective segment, particularly longer in segments 6 and 7. Pleons not immersed in pereons, pleonites decreasing in width towards posterior, ventro-lateral margins not expanded posteriorly. Pleonites more uniformly pigmented than pereonites.

Cephalon bluntly sagittal, eyes large, well developed, oblong and set wide apart. Stippled pigmentation is dense between eyes. Antenna (eight segments) longer than antennule (eight segments), rounded, bases set wide apart. Pereopods robust, without expanded carina on basis. All pereopods with large, smooth dactylus, densely pigmented at apex. Merus and carpus of pereopods 1-3 shorter than propodus and ischium. Prodopodus 1-3 arched anteriorly, Prodopodus 4-7 arched posteriorly. Distoventral margin of basis had a line of chromatophores. Pleopods without setae, but pleopods 2-5 branchiated. Pleopods with plumose marginal setae. Pleotelson with denser pigmentation anteriorly, distal margin round with uropod rami extending beyond posterior margin. Uropod exopods thinner than endopods, endopod apex offset, both uropod apices bluntly rounded. Uropod, exopod and endopod possessed plumose marginal setae (Table 1, Figures 3, 4 and 5).

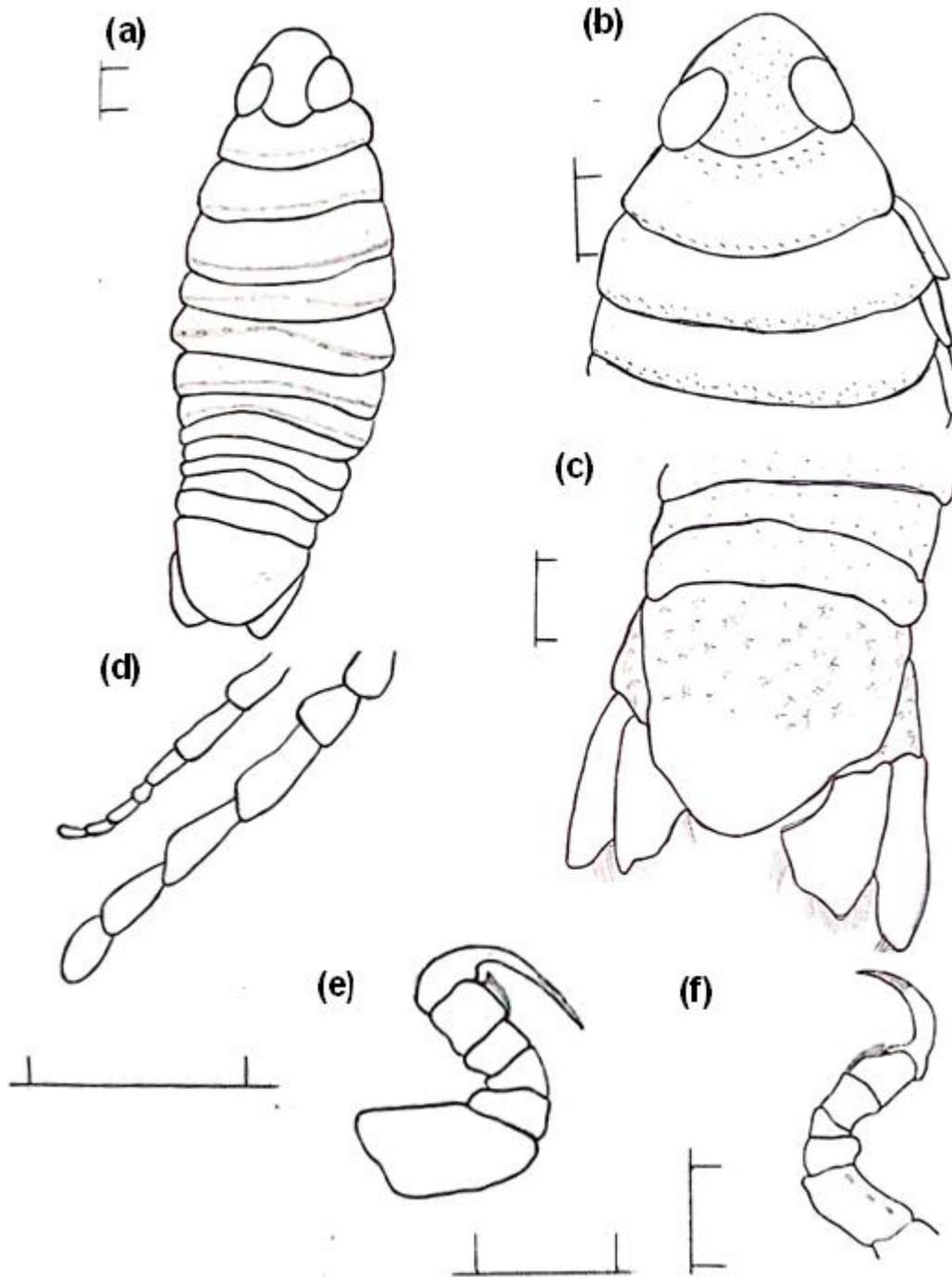
**Table 1.** Biometrics of *L. redmanii* collected from Lake Qaroun, Egypt.

Parameters (mm)/sex	Males	Female
Total body L.	16.6 ± 1.2	15
Total body W.	5.7 ± 0.4	6.6
Cephalon L.	1.9 ± 0.2	2.3
Eye L.	1.2 ± 0.3	0.9
Eye W.	0.6 ± 0.2	0.4
Eye/cephalon L. ratio	0.6	3.0
Antennule L.	1.6 ± 0.2	1.4
Antennae L.	2.4 ± 0.2	2.0
Telson L.	3.3 ± 0.1	3.0
Uropod L.	3.6 ± 0.1	3.5

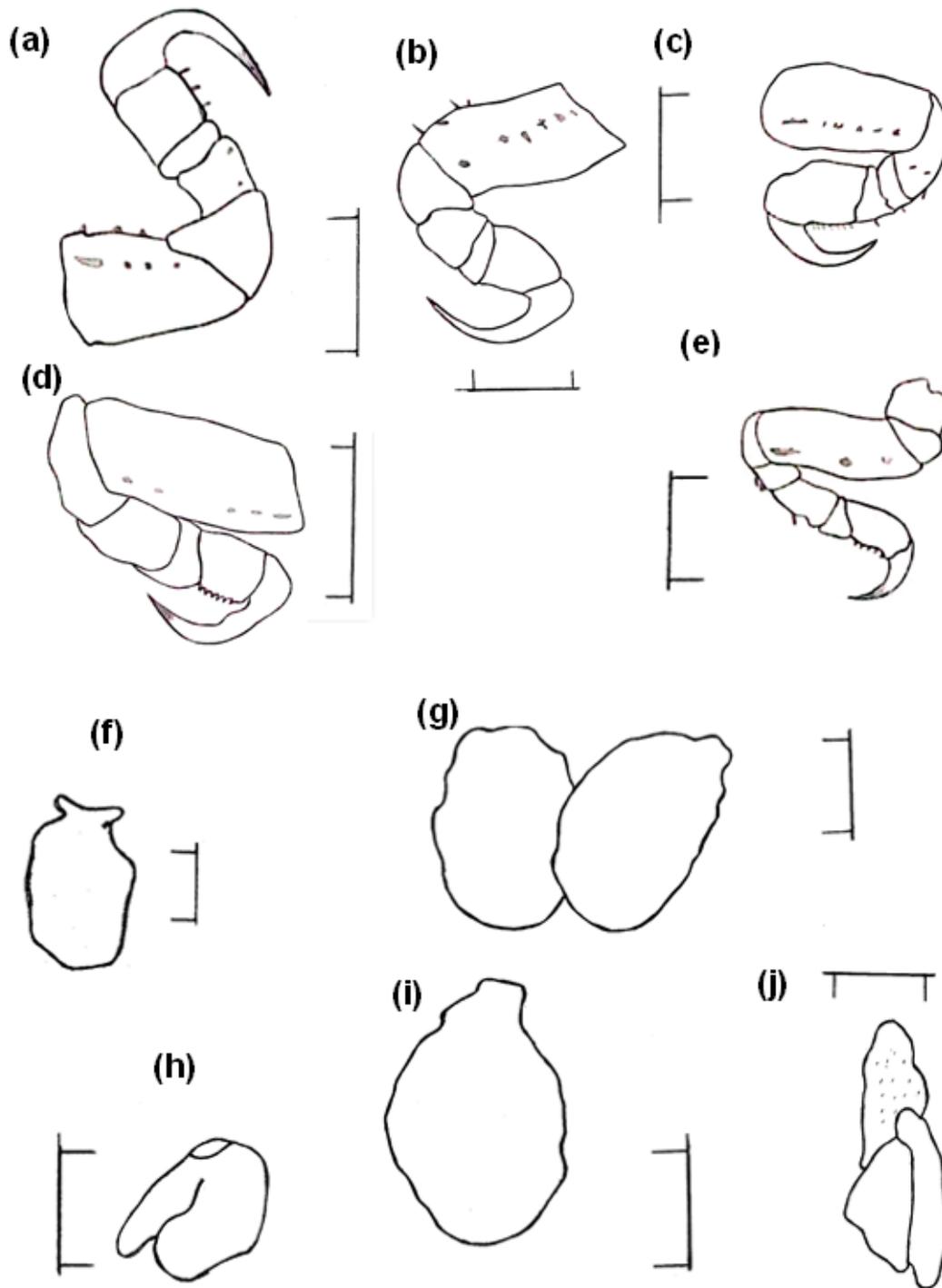
Note, values are means ± SD, *n* = 10 males and 1 female. L, length and W, width.



**Fig. 3:** *L. redmanii* female: (a) dorsal view; (b) enlarged anterior dorsal view showing pigmentation; (c) enlarged posterior dorsal view; (d) pereopod 1; (e) pereopod 2; (f) pereopod 3; (g) pereopod 6; (h) pereopod 7. Scale bar; 1 mm.



**Fig. 4:** *L. redmanii* male: (a) dorsal view; (b) enlarged anterior dorsal view; (c) enlarged posterior dorsal view; (d) antenna and antennule; (e) pereopod 1; (f) pereopod 2. Scale bar; 1 mm.



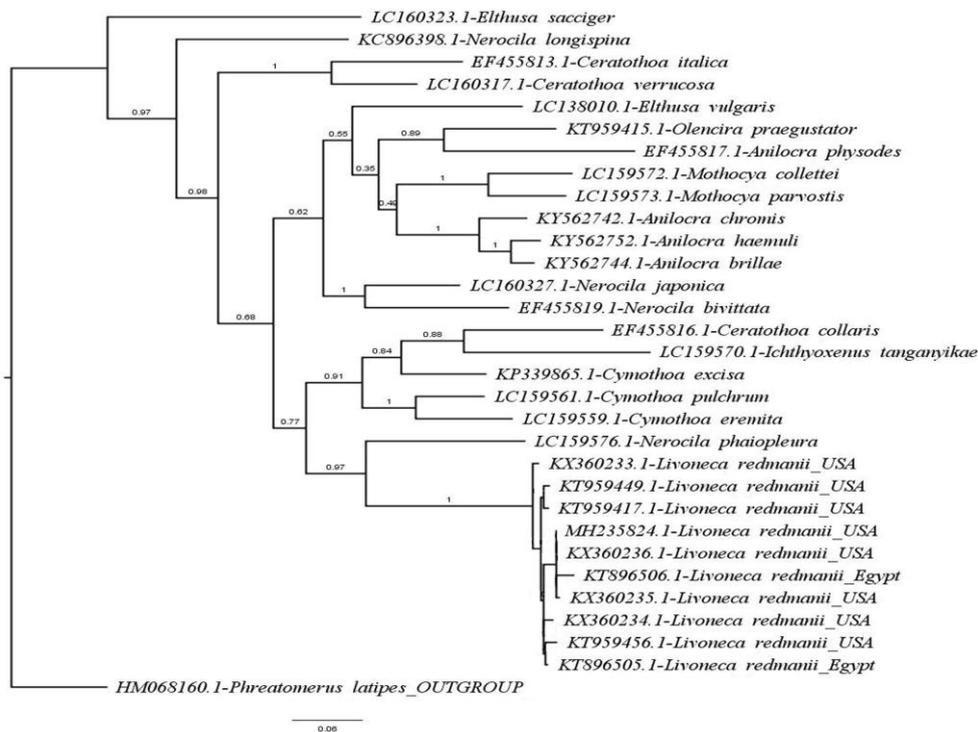
**Fig. 5:** *L. redmanii* male: (a) pereopod 3; (b) pereopod 4; (c) pereopod 5; (d) pereopod 6 (e) pereopod 7; (f) pleopod 1; (g) pleopod 2 (h) pleopod 3; (I) pleopod 4; (j) uropod. Scale bar; 1 mm.

### Sexual dimorphism of *L. redmanii* from Lake Qaroun

Body of the males was more slender than the female, and more fusiform in shape. Eyes ommatidia were obscured by exoskeleton in female and unobscured by exoskeleton in males. In comparison to female, males' eyes much larger but females with greater eye/cephalaon length ratio. Female had longer cephalon than males, but shorter antennae. Males' cephalon had darker and denser pigmentation than the sole female examined. Males' last four segments of antennules are aesthetasces. Male pereopods had spinoise setae, number of setae decreasing proximally, with smooth dactylus. However, female pereopods smooth, lacking stout spines. Female had shorter telson and uropods than males (Table 1, Figures 3, 4 and 5).

### Sequence analyses

The present study successfully sequenced a partial fragment of the *COI* gene in all 10 Lake Qaroun isopods. From these ten sequences, we recovered two haplotypes: Hap1 (KT896505.1), which comprised 90% of the samples (n=1) and Hap2 (KT896506.1), which comprised 10% (n=1) of the samples, with a one base pair difference between the two haplotypes. The Jukes-Cantor (JC69) and Felsenstein (F81) were the best-fitted evolutionary models selected for the calculation of *p*-distance and phylogenetic analysis, respectively. Both Egyptian haplotypes matched with high percent identifications (BLAST: >99.5%) to *COI* sequences obtained from three wild-caught *L. redmanii* from Chesapeake Bay, Maryland, USA. Moreover, the *COI p*-distances within the Egyptian and American isopods identified as *L. redmanii* were low (0-0.017; Table 2). Conversely, *COI p*-distances between *L. redmanii* and remaining isopods included in the present study were markedly higher (0.256-0.436; Table 2). The Egyptian and American isopods identified as *L. redmanii* formed a clade with high node support (BI 1; Figure 6.).



**Fig. 6:** Phylogeny estimated using a Bayesian Inference approach, with the F81 model of nucleotide evolution for *Livoneca redmanii* and selected cymothoid isopods.

**Table 2:** Interspecific and intraspecific uncorrected pairwise distances at *COI* among parasitic isopod species.

Species	KT896505	KT896506	KX360233	KT959417	MH235824	KX360235	KX360236	KT959456	KT959449	KX360234	KY562742	KY562742	EF455817	EF455816	EF455813	LC160317	LC159559	LC159576	LC159572	EF455819	LC159573	LC159571	LC160327	KG965398	LC159575	KT969415	HM068160	
KT896505.1-Livoneca redmanii-hap1 Egypt																												
KT896506.1-Livoneca redmanii-hap2 Egypt	0.012																											
KX360233.1-Livoneca redmanii-USA	0.005	0.017																										
KT959417.1-Livoneca redmanii-USA	0.000	0.012	0.005																									
MH235824.1-Livoneca redmanii-USA	0.000	0.012	0.005	0.000																								
KX360235.1-Livoneca redmanii-USA	0.000	0.012	0.005	0.000	0.000																							
KX360236.1-Livoneca redmanii-USA	0.000	0.012	0.005	0.000	0.000	0.000																						
KT959456.1-Livoneca redmanii-USA	0.000	0.012	0.005	0.000	0.000	0.000	0.000																					
KT959449.1-Livoneca redmanii-USA	0.000	0.012	0.005	0.000	0.000	0.000	0.000	0.000																				
KX360234.1-Livoneca redmanii-USA	0.000	0.012	0.005	0.000	0.000	0.000	0.000	0.000	0.000																			
KY562742.1-Anilocra chromis	0.342	0.358	0.342	0.342	0.342	0.342	0.342	0.342	0.342	0.342	0.342	0.342	0.342	0.342	0.342	0.342	0.342	0.342	0.342	0.342	0.342	0.342	0.342	0.342	0.342	0.342	0.342	0.342
KY562742.1-Anilocra brillae	0.335	0.346	0.335	0.335	0.335	0.335	0.335	0.335	0.335	0.335	0.335	0.335	0.335	0.335	0.335	0.335	0.335	0.335	0.335	0.335	0.335	0.335	0.335	0.335	0.335	0.335	0.335	0.335
KY562742.1-Anilocra haemuli	0.339	0.354	0.339	0.339	0.339	0.339	0.339	0.339	0.339	0.339	0.339	0.339	0.339	0.339	0.339	0.339	0.339	0.339	0.339	0.339	0.339	0.339	0.339	0.339	0.339	0.339	0.339	0.339
EF455817.1-Anilocra physodes	0.331	0.350	0.331	0.331	0.331	0.331	0.331	0.331	0.331	0.331	0.331	0.331	0.331	0.331	0.331	0.331	0.331	0.331	0.331	0.331	0.331	0.331	0.331	0.331	0.331	0.331	0.331	0.331
EF455816.1-Ceratothoa collaris	0.309	0.320	0.305	0.309	0.309	0.309	0.309	0.309	0.309	0.309	0.309	0.309	0.309	0.309	0.309	0.309	0.309	0.309	0.309	0.309	0.309	0.309	0.309	0.309	0.309	0.309	0.309	0.309
EF455813.1-Ceratothoa italica	0.374	0.382	0.378	0.374	0.374	0.374	0.374	0.374	0.374	0.374	0.374	0.374	0.374	0.374	0.374	0.374	0.374	0.374	0.374	0.374	0.374	0.374	0.374	0.374	0.374	0.374	0.374	0.374
LC160317.1-Ceratothoa verrucosa	0.316	0.331	0.312	0.316	0.316	0.316	0.316	0.316	0.316	0.316	0.316	0.316	0.316	0.316	0.316	0.316	0.316	0.316	0.316	0.316	0.316	0.316	0.316	0.316	0.316	0.316	0.316	0.316
LC159559.1-Cymothoa eremita	0.287	0.301	0.291	0.287	0.287	0.287	0.287	0.287	0.287	0.287	0.287	0.287	0.287	0.287	0.287	0.287	0.287	0.287	0.287	0.287	0.287	0.287	0.287	0.287	0.287	0.287	0.287	0.287
KF339665.1-Cymothoa excisa	0.259	0.269	0.256	0.259	0.259	0.259	0.259	0.259	0.259	0.259	0.259	0.259	0.259	0.259	0.259	0.259	0.259	0.259	0.259	0.259	0.259	0.259	0.259	0.259	0.259	0.259	0.259	0.259
LC159561.1-Cymothoa pulchrum	0.262	0.276	0.262	0.262	0.262	0.262	0.262	0.262	0.262	0.262	0.262	0.262	0.262	0.262	0.262	0.262	0.262	0.262	0.262	0.262	0.262	0.262	0.262	0.262	0.262	0.262	0.262	0.262
LC160323.1-Elthusa sacciger	0.428	0.436	0.432	0.428	0.428	0.428	0.428	0.428	0.428	0.428	0.428	0.428	0.428	0.428	0.428	0.428	0.428	0.428	0.428	0.428	0.428	0.428	0.428	0.428	0.428	0.428	0.428	0.428
LC138010.1-Elthusa vulgaris	0.305	0.324	0.301	0.305	0.305	0.305	0.305	0.305	0.305	0.305	0.305	0.305	0.305	0.305	0.305	0.305	0.305	0.305	0.305	0.305	0.305	0.305	0.305	0.305	0.305	0.305	0.305	0.305
LC159570.1-Ichthyoxenus tanganyikae	0.350	0.354	0.354	0.350	0.350	0.350	0.350	0.350	0.350	0.350	0.350	0.350	0.350	0.350	0.350	0.350	0.350	0.350	0.350	0.350	0.350	0.350	0.350	0.350	0.350	0.350	0.350	0.350
LC159572.1-Mothocya collettei	0.335	0.342	0.331	0.335	0.335	0.335	0.335	0.335	0.335	0.335	0.335	0.335	0.335	0.335	0.335	0.335	0.335	0.335	0.335	0.335	0.335	0.335	0.335	0.335	0.335	0.335	0.335	0.335
LC159573.1-Mothocya panostis	0.309	0.316	0.305	0.309	0.309	0.309	0.309	0.309	0.309	0.309	0.309	0.309	0.309	0.309	0.309	0.309	0.309	0.309	0.309	0.309	0.309	0.309	0.309	0.309	0.309	0.309	0.309	0.309
EF455819.1-Nerocila bivittata	0.309	0.324	0.312	0.309	0.309	0.309	0.309	0.309	0.309	0.309	0.309	0.309	0.309	0.309	0.309	0.309	0.309	0.309	0.309	0.309	0.309	0.309	0.309	0.309	0.309	0.309	0.309	0.309
LC160327.1-Nerocila japonica	0.262	0.280	0.262	0.262	0.262	0.262	0.262	0.262	0.262	0.262	0.262	0.262	0.262	0.262	0.262	0.262	0.262	0.262	0.262	0.262	0.262	0.262	0.262	0.262	0.262	0.262	0.262	0.262
KG965398.1-Nerocila longispina	0.346	0.350	0.346	0.346	0.346	0.346	0.346	0.346	0.346	0.346	0.346	0.346	0.346	0.346	0.346	0.346	0.346	0.346	0.346	0.346	0.346	0.346	0.346	0.346	0.346	0.346	0.346	0.346
LC159576.1-Nerocila phaeopleura	0.283	0.301	0.283	0.283	0.283	0.283	0.283	0.283	0.283	0.283	0.283	0.283	0.283	0.283	0.283	0.283	0.283	0.283	0.283	0.283	0.283	0.283	0.283	0.283	0.283	0.283	0.283	0.283
KT959415.1-Olencira praegustator	0.301	0.320	0.298	0.301	0.301	0.301	0.301	0.301	0.301	0.301	0.301	0.301	0.301	0.301	0.301	0.301	0.301	0.301	0.301	0.301	0.301	0.301	0.301	0.301	0.301	0.301	0.301	0.301
HM068160.1-Phreatomerus latipes	0.362	0.382	0.358	0.362	0.362	0.362	0.362	0.362	0.362	0.362	0.362	0.362	0.362	0.362	0.362	0.362	0.362	0.362	0.362	0.362	0.362	0.362	0.362	0.362	0.362	0.362	0.362	0.362

**Literature review**

The majority of isopods identified as species other than *L. redmanii* in Lake Qaroun were determined to be *L. redmanii* after an examination of the previously published photographs and illustrations (Table 3). Reports of *A. physodes* (Shaheen *et al.*, 2017), *Nerocila orbigny* (Abdel-Latif, 2016; Mahmoud *et al.*, 2016; Younes *et al.*, 2016; Shaheen *et al.*, 2017) and *Nerocila thresherorum* (Mahmoud *et al.*, 2016, 2017; Ali and Aboyadak, 2018) represent *L. redmanii*. Additionally, unidentified “Isopoda” presented in Elgendy *et al.* (2017) were also *L. redmanii*. Reports of *L. redmanii* in Lake Qaroun were corroborated for Helal and Yousef (2018), Mahmoud *et al.* (2017 and 2019) and Shaheen *et al.* (2017); reported as the junior synonym, *L. ovalis*). The only other cymothoid species besides *L. redmanii* noted in Lake Qaroun was an unidentified *Anilocra* sp., which was reported as *A. physodes* in Mahmoud *et al.* (2017).

The present study determined that reports of *R. thresherorum* in the Suez Canal (Youssef *et al.*, 2014) and *A. leptosoma* in coastal Egypt (Rania and Rehab, 2015) also represent *L. redmanii*. Additional studied suggested misidentifications include *A. meridionalis* in the Suez Canal (Youssef *et al.*, 2014), which possibly represented two species of unidentified *Anilocra*; *C. exigua* in the Suez Canal, which represented an unidentified *Cymothoa*; and *C. indica* from coastal Egypt (Rania and Rehab, 2015), which represented a species of *Elthusa*.

**Table 3:** Corrected identifications of cymothoids collected from Lake Qaroun, Suez Canal and Mediterranean Sea. Identifications produced after consultations with Niel Bruce (Queensland Museum, Australia). Reports of *L. redmanii* are deemed correct in Helal and Yousef (2018) and Mahmoud *et al.* (2017, 2019). <sup>1</sup> *Livoneca ovalis* is recognized as a junior synonym of *Livoneca redmanii*.

Published name	Source	Corrected name
<i>Anilocra leptosoma</i>	Rania and Rehab (2015)	<i>Livoneca redmanii</i> Leach, 1818
<i>Anilocra physodes</i>	Mahmoud <i>et al.</i> (2017)	<i>Anilocra</i> sp.
<i>Anilocra physodes</i>	Shaheen <i>et al.</i> (2017)	Immature? <i>Livoneca redmanii</i> Leach, 1818
<i>Anilocra meridionalis</i>	Youssef <i>et al.</i> (2014)	<i>Anilocra</i> sp. (possibly two species)
<i>Cymothoa exigua</i>	Youssef <i>et al.</i> (2014)	<i>Cymothoa</i> sp.
<i>Cymothoa indica</i>	Rania and Rehab (2015)	<i>Elthusa</i> sp.
<i>Livoneca redmanii</i>	Helal and Yousef (2018)	<i>Livoneca redmanii</i> Leach, 1818
<i>Livoneca ovalis</i> <sup>1</sup>	Shaheen <i>et al.</i> (2017)	<i>Livoneca redmanii</i> Leach, 1818
<i>Nerocila orbignyi</i>	Abdel-Latif (2016)	<i>Livoneca redmanii</i> Leach, 1818
<i>Nerocila orbignyi</i>	Mahmoud <i>et al.</i> (2016)	Immature? <i>Livoneca redmanii</i> Leach, 1818
<i>Nerocila orbignyi</i>	Shaheen <i>et al.</i> (2017)	<i>Livoneca redmanii</i> Leach, 1818
<i>Nerocila orbignyi</i>	Younes <i>et al.</i> (2016)	<i>Livoneca redmanii</i> Leach, 1818
<i>Renocila thresherorum</i>	Youssef <i>et al.</i> (2014)	<i>Livoneca redmanii</i> Leach, 1818
<i>Renocila thresherorum</i>	Mahmoud <i>et al.</i> (2016)	<i>Livoneca redmanii</i> Leach, 1818
<i>Renocila thresherorum</i>	Mahmoud <i>et al.</i> (2017)	<i>Livoneca redmanii</i> Leach, 1818
<i>Renocila thresherorum</i>	Shaheen <i>et al.</i> (2017)	<i>Livoneca redmanii</i> Leach, 1818
<i>Renocila thresherorum</i>	Ali and Aboyadak (2018)	<i>Livoneca redmanii</i> Leach, 1818
<b>Isopoda</b>	Elgendy <i>et al.</i> (2017)	<i>Livoneca redmanii</i> Leach, 1818

## DISCUSSION

The present molecular and morphological analyses strongly support the identification of cymothoid isopods collected in Lake Qaroun as the American species *L. redmanii*. An Egyptian female isopod closely matched Bruce's (1990) figures of *L. redmanii* (which included the holotype) and was morphologically similar to *L. redmanii* from the mid-Atlantic US and males were morphologically similar to Bakenhaster's (2004) description of early natatory stage *L. redmanii* from the US Gulf of Mexico. Additionally, American and Egyptian *L. redmanii* comprised a single clade with strong node support, possessed low intraspecific *COI* p-distances, and BLAST produced high percent identifications scores (>99%) among sequences.

At present, only two cymothoid species have been confirmed in Lake Qaroun, Egypt: *L. redmanii* and an unidentified juvenile *Anilocra*. Previously published reports of *A. physodes* (Shaheen *et al.*, 2017), *N. orbignyi* (Abdel-Latif, 2016; Mahmoud *et al.*, 2016; Younes *et al.*, 2016; Shaheen *et al.*, 2017) and *R. thresherorum* (Mahmoud *et al.*, 2016, 2017; Shaheen *et al.*, 2017; Ali and Aboyadak, 2018) could not be corroborated - given body shape, coloration, attachment locations, pleotelson shape, and arrangement of the antennae/antennules, pereonites, pleonites, and uropod endopods and exopods - and represent misidentifications of *L. redmanii*. While a figure of *A. physodes* presented in Mahmoud *et al.* (2017) could only be verified at the generic level, a species of *Anilocra* is present in Lake Qaroun, which could reflect *A. physodes*, a cymothoid that occurs widely in the Mediterranean. Additionally, the reports of *R. thresherorum* from Suez Canal could not be corroborated, and this species should not be considered invasive in Afro-Eurasia at present, as we are unaware of any confirmed occurrences of *R. thresherorum* outside

of its native range (Baja California, Mexico). Two further species not native to the Mediterranean or Indo-Pacific (*A. meridionalis* and *C. exigua*) have also been reported from the nearby Suez region (Youssef *et al.*, 2014). The photographs of *A. meridionalis* are not compatible with that species and the specimens illustrated are of an unidentified *Anilocra*, possible two species given the different body shapes shown (N.L. Bruce, personal communication). The figures of *C. exigua* were only verified at the generic level. Additionally, the figures of *A. leptosoma* from coastal Egypt presented in Rania and Rehab (2015) are more consistent with *L. redmanii*. We are unaware of any verified reports of the *A. meridionalis* in the Mediterranean/Indo-Pacific or *A. leptosoma* or *C. exigua* in the Mediterranean Sea, and these species should not be considered invasive in those regions in the absence of additional data. However, *C. exigua* has been reported from the Red Sea off the coast of Yemen (Al-Zubaidy and Mhaisen, 2013). Although the line drawings of *N. orbigny* and *R. thresherorum* presented in Mahmoud *et al.* (2016) probably support the given identifications, direct comparison between the figures and drawings show clear inconsistencies (N.L. Bruce, personal communication). While cymothoids may be difficult to identify at species level for non-taxonomists (Horton, 2000), recent revisions (e.g. Bruce, 1987a, b, c, 1987; Hadfield *et al.*, 2013; Martin *et al.*, 2016) allow for relatively straightforward identification at generic level. The validation of *Nerocila* and *Renocila* species in Egypt requires further evidence (N.L. Bruce, personal communication).

In addition, specific characters were reported in the cymothoid isopod, *L. redmanii* collected from Lake Qaroun, Egypt. Spines on pereopods (3<sup>rd</sup> – 7<sup>th</sup>) of males on the first 4 segments or all segments depending on the pereopod location were reported. Similarly, spines on the first 4 segments of pereopods in *L. redmanii* manca were reported by Bakenhaster (2004). Moreover, characteristic pigmentations patterns on the isopod's cephalon, pereonites, pleonites and pereopods were specific to *L. redmanii*. Sinistral bending of our specimens' bodies also was characteristic to *L. redmanii* (Florida, Poulakis *et al.*, 2010). The differences between female and male biometrics, pigmentation, eye covering and pereopods (spines) can be attributed to morphology adaptations of the parasite to fit in the host. Males usually attach to the gills/skin (pereopods with spines) and females usually migrate to the buccal cavity and attach to the tongue (no pereopods' spines in the present results) and also to act as chitinous armour inside the fish's buccal cavity (see Brusca and Gilligan, 1983). The attachment locations also differ between *L. redmanii* (gill chamber) and these three species (exterior skin/fins). Furthermore, our phylogenetic analyses included sequences of *A. physodes* [(collected in Italy (Ketmaier *et al.*, 2008)], which were markedly dissimilar to the Egyptian and American *L. redmanii*. Mitochondrial *COI* sequences were unavailable for *N. orbigny* and *R. thresherorum*.

Although *L. redmanii* was first noted in Lake Qaroun in 2015, our corrected identifications indicate that it was present in the Mediterranean/Suez Canal as early as 2013/2014. Although cymothoid isopods are well represented in museums collections from the mid-1800s (Smit *et al.*, 2014), no Old World records exist for *L. redmanii* (or associated junior synonyms). Though, more exhaustive review of the literature may unearth additional misidentifications. While possible, it is unlikely that *L. redmanii* was transported to the Mediterranean region via natural fish movement, as few of the reported fish hosts are transatlantic migrants. It is far more probable that *L. redmanii* is a recent Afro–Eurasian invader by anthropogenic means, likely transported into the Mediterranean region in the ballast water of ships and then transferred secondarily into Lake Qaroun. The larval period (1–2 weeks; Sandifer and

Kerby, 1983) is within the transit time of vessels traveling between Eastern US and Mediterranean ports. Moreover, infected host fishes may be able to survive inside ballast tanks for extended periods of time (Whitman Miller, Smithsonian Environmental Research Center, personal communication), and produce larvae that could be introduced into non-native waters during ballast water exchanges. The Mediterranean is one of the most heavily invaded marine systems (Edelist *et al.*, 2013) and ballast water is the suspected introduction vector for a number of species, such as the ctenophore *Mnemiopsis leidyi* (Ghabooli *et al.*, 2013), a species sympatric with *L. redmanii* in most of their native ranges. *M. leidyi* was first detected in the Black Sea in the early 1980s and has spread throughout the Mediterranean by natural dispersal and ballast water re-introductions (Fuentes *et al.*, 2010). In 2013, *M. leidyi* was reported in Lake Qaroun and Wadi El Rayan II, a lower salinity inland lake located ~25km southwest of Lake Qaroun (El-Shabrawy and Dumont, 2016).

For nearly a century, Lake Qaroun has been stocked with larval fish obtained from local coastal waters and the Nile River estuary, which has inadvertently introduced several non-target fish and invertebrate species (Ishak, 1980; El-Serafy *et al.*, 2014; El-Shabrawy and Dumont, 2016). These stocking events represent a likely vector for the introduction of *L. redmanii* into Lake Qaroun, either directly or co-introduced with infected hosts. Similarly, it was suggested that these stocking events were the likely pathway for the introduction of the invasive ctenophore *M. leidyi* into Lake Qaroun and Wadi El Rayan II in 2013 (El-Shabrawy and Dumont, 2016). The present study recommend that stockings be screened as much as possible to limit continued introductions of cymothoid isopods, *M. leidyi* or other unwanted species into in Lake Qaroun or other Egyptian inland lakes. Cymothoids can settle on non-target hosts, such as gelatinous zooplankton, cephalopods, poriferans, and elasmobranchs (Trilles and Öktener, 2004; Ateş *et al.*, 2006) or fisheries gear, such as seines and nets (Aguilar, personal observation). Thus, care should be taken when transferring fishery resources or gear among systems in the region. Given host specificities, shifting to stocking shrimp in Lake Qaroun for a period of time could reduce the number of fish hosts and reduce the likelihood of introducing additional fish-infecting cymothoids, though care should be taken to not also inadvertently release free-swimming cymothoids along with the target shrimp species.

The introduction of *L. redmanii* into the Mediterranean Sea proper, Suez Canal, and Lake Qaroun represents the third confirmed cymothoid species to invade the Mediterranean. The other species include *C. indica*, a Lessepsian migrant (Trilles and Bariche, 2006) and *Anilocra pilchardi* Bariche and Trilles (2006), described from Lebanon, but considered a Lessepsian migrant due to membership in the Indo-Pacific 'alloceraea group' (Bariche and Trilles, 2006). *Ceratothoa collaris* Schioedte and Meinert, 1883 is considered new to eastern Mediterranean (Lebanon; Bariche and Trilles, 2008; Shakman *et al.*, 2009), but has historically ranged from the eastern Atlantic (Senegal) to western Mediterranean (Tunisia). Although *N. orbigny* has been reported from the south-west Pacific, Indian Ocean, Mediterranean Sea, and eastern Atlantic (Bruce, 1987c; Kensley, 2001; Öktener and Trilles, 2004), the type locality is Greece and it is considered native to the Mediterranean region. A rigorous survey of Mediterranean cymothoids is needed to determine the range of *L. redmanii* in the region and better understand native and invasive cymothoid biogeography.

The massive damage and mortalities of Lake Qaroun fishes associated with *L. redmanii* (and possibly other isopods species) infection are atypical for this species. Within its native range, the physiological cost of *L. redmanii* infection appears to be small and may not constitute a threat to individual survival (Landau *et al.*, 1995;

Marks *et al.*, 1996). The severity of *L. redmanii* infections in Lake Qaroun in relation to those noted in its native range likely resulted from a decrease in predation pressure/natural mortality rates afforded in the new habitat; genetic differences between native and novel host species; physiological condition of host species, the majority of which are not native to Lake Qaroun (and many of those are unable to naturally reproduce in the lake); possible multi-species cymothoid infections; density of fish hosts and the hydrology and physio-chemical characteristics of the closed Lake Qaroun, which can experience high pollutant levels, and possible concurrent bacterial infections (Vignon and Sasal, 2010; Peeler *et al.*, 2011; Lymbery *et al.*, 2014; Elgendy *et al.*, 2017). Although one of the main fish hosts (*T. zillii*) has acclimated to increased salinities in Lake Qaroun (being naturally a freshwater species), it may still be under physiological stress, which may increase the susceptibility of infection or subsequent reaction to infection by a novel parasite. While we have no quantitative data on *L. redmanii* abundance in Lake Qaroun at present, cymothoid infection rates  $\geq 80\%$  have been reported (Mahmoud *et al.*, 2017; Shaheen *et al.*, 2017), much higher than for *L. redmanii* in its native range (Lindsay and Moran, 1976; Landau *et al.*, 1995; Aguilar, personal observation).

Fishery stocks in Lake Qaroun may have also been negatively impacted by predation from *M. leidy*, which was introduced around the same timeframe as *L. redmanii* (El-Shabrawy and Dumont, 2016). This ctenophore is a voracious predator of zooplankton, including small crustaceans and fish eggs and larvae (Purcell *et al.*, 2001). Additionally, Lake Qaroun can experience prolonged periods of high water temperatures ( $\sim 40^\circ\text{C}$ ) during the summer months and the main source of freshwater input is from agricultural runoff (El-Shabrawy and Dumont, 2016). Further work is needed to better understand *L. redmanii* and *M. leidy* population dynamics, parasite-host dynamics, food web dynamics and other factors that may impact fish populations in Lake Qaroun. This is particularly important because changes in the fish populations and prey resources in Lake Qaroun could negatively affect resident and migratory bird abundances and alter movement patterns, adding a multiple stressor to local economies dependent on commercial fisheries and ecotourism.

At present, *L. redmanii* is the senior accepted name for this species (see Bruce [1990] and Trilles [1994]). Prior to synonymy, the Chesapeake Bay and Egyptian cymothoid samples would have been classified as *L. ovalis*. *L. redmanii sensu lato* has a large range (Massachusetts, along the eastern US through the Caribbean to South America; Sandifer and Kerby, 1983; Bruce, 1990; Williams and Bunkley-Williams, 1996; De Lima *et al.*, 2005) can be found in estuarine (including lower salinity areas) and coastal/offshore habitats (Lindsay and Moran, 1976; Landau *et al.*, 1995; Aguilar, personal observation); and has a varied array of fish hosts, from nearshore residents to highly-migratory anadromous and marine fishes (Alperin, 1966; Lindsay and Moran 1976; Landau *et al.*, 1995; dos Santos Costa and Chellappa, 2010). Williams and Bunkley-Williams (1996) supported the separation of *L. redmanii* and *L. ovalis* and noted that *L. redmanii* occurs in male–female pairs, only infects the marine fish species Cero *Scomberomorus regalis* and Serra Spanish Mackerel *S. brasiliensis* (though more recently it has also been reported from the carangid, Atlantic Bumper *Chloroscombrus chrysurus* in Brazil; dos Santos Costa and Chellappa, 2010), and ranges from the Caribbean to Brazil, whereas *L. ovalis* occurs singly with females not paired with males (which raises some questions about reproductive strategy), infects fishes from coastal freshwaters to the open ocean, but more commonly occurs in estuarine and inshore areas, and ranges along the US Atlantic and Gulf coasts. Thus, there may be some ecological differences among certain populations of *L. redmanii*

*sensu lato*, which may reflect a species complex. Leach (1818) did not mention fish hosts or male-female coinfection in the *L. redmanii* original description and we are unaware if any correspondence with the collector M.R. Redmond is extant. Although there may be some minor morphological differences between the *L. redmanii* holotype and US individuals (N.L. Bruce, personal communication), the isopods examined in the present study confirmed well with Bruce's (1990) examination of *L. redmanii*. A detailed analysis of *L. redmanii sensu lato* from across its entire range, preferably employing an integrative taxonomic approach (i.e., incorporating morphological, genetic, and ecological data), is required to fully resolve taxonomic status, but is beyond the scope of the present study. Notwithstanding, in order to revalidate *L. ovalis*, a neotype designation is warranted, which may be challenging given uncertainties surrounding the availability or existence type material (N.L. Bruce, personal communication).

## CONCLUSION

Genetic barcoding used in conjunction with traditional morphological analyses were powerful tools in confirming the identity of an invasive isopod (*L. redmanii*) that was suspected in causing tremendous damage to commercial fisheries in Lake Qaroun, Egypt. We strongly recommend fishery managers exercise caution when stocking Lake Qaroun and other inland lakes with fish or shrimp collected from nearby coastal and estuarine systems to reduce the likelihood of further introductions of *L. redmanii* or other non-native species. Similar care should be taken if gear or fishery resources are transferred among adjacent inland lakes. We also recommend that fishery-independent surveys and commercial fishers within Egypt and the Mediterranean/Red Sea region record any possible occurrences, particularly as *L. redmanii* may superficially resemble other cymothoid parasites, to track the spread of this invasive species. Finally, a rigorous taxonomic analysis of *L. redmanii sensu lato* from across its entire range is needed to resolve longstanding taxonomic uncertainties surrounding this species.

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## ARABIC SUMMARY

تأكيد جزيني ومورفولوجي لمتشابه الأرجل الأمريكي الغازي *Livoneca redmanii* Leach, 1818، من منطقة البحر الأبيض المتوسط الي بحيرة قارون، مصر

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  - ٤- مكتب التقنيات الحديثة لتربية الأحياء البحرية، القاهرة، مصر.
  - ٥- الوكالة المصرية للطاقة الذرية، القاهرة، مصر.

تم العثور على طفيليات مجهولة من متشابهة الأرجل تتبع عائلة Cymothoidae والتي كانت مرتبطة بوفيات الأسماك الكبيرة في بحيرة قارون؛ البحيرة المغلقة عالية الملوحة في شمال وسط مصر، في عام ٢٠١٥، وقد تسببت هذه الطفيليات في إصابة مجموعة كبيرة من الأسماك المضيفة المهمة تجارياً، ومنها *Liza spp*, *Mugil spp.*, *Solea solea*, *Dicentrarchus labrax*, *Tilapia zillii* من الأبحاث الحديثة في مجملها انه يوجد أربعة أنواع من طفيليات عائلة Cymothoidae في بحيرة قارون، ومع ذلك، فإن العديد من الصور الفوتوغرافية والرسوم التوضيحية المقدمة كانت غير دقيقة على مستويات الجنس والنوع. تم تجميع عينات من متشابهات الأرجل من أسماك البلطي النيلي من قرية شكشوك على بحيرة قارون، قد استخدمت هذه الدراسة نهجاً تصنيفياً متكاملاً يتضمن ويعتمد على طرق التصنيف التقليدية وبيانات التنشيف اللوحي الجيني بسلسلة جين COI الميتوكوندري والعلاقات الجينية النسبية لتحديد نوع عينات متشابهة الأرجل التي تم جمعها من بحيرة قارون. تم تعريف جميع متشابهة الأرجل التي تم فحصها على أنها *Livoneca redmanii* Leach, 1818، والتي تنتمي للمصبات والمياه البحرية بغرب المحيط الأطلسي، والتي تطابقت بنسبة ١٠٠% جينياً ونسبياً مع مثيلاتها التي تم تجميعها من Chesapeake Bay بشرق الولايات المتحدة الأمريكية. تميز هذا النوع من حيث الشكل الخارجي بالتصبغ على السيفالون، البريونايت، البليوننايت، التيلسون، البيروبودس و يكون هذا النوع منحنياً قليلاً إلى اليسار. وخلصت مراجعة الأبحاث الحديثة ان غالبية cymothoids في بحيرة قارون والمناطق البحرية المجاورة والذين تم تحديدهم كأصناف أخرى غير *L. redmanii* كانوا في الواقع *L. redmanii*، او مراحل مبكرة من متشابهة الأرجل من جنس *Anilocra*، أو أنواع غير مُعرفة من عائلة Cymothoidae، وبالمثل فإن *L. redmanii* يبدو انه هو النوع الموجود في شرق البحر الأبيض المتوسط وقناة السويس، ومن المحتمل أن يكون حامل متشابهة الأرجل الذي تم إدخاله إلى بحيرة قارون قادماً مع النقل المستمر للأسماك من مصب البحر الأبيض المتوسط ونهر النيل إلى بحيرة قارون (كما حدث بالفعل مع أحد الأنواع الأمريكية الغازية التي ظهرت في البحيرة وهو قنديل البحر *Mnemiopsis leidyi*). ويمكن أن تساعد هذه الدراسة المسؤولين عن المصايد على فهم ديناميكيات الطفيليات و عوائلها بشكل أفضل، وتطوير استراتيجيات مكافحة الحيوية للتخفيف من أثارها في بحيرة قارون. ومن ثم الحد من انتشارها بين أنظمة البحيرات المجاورة ومصبات الأنهار والسواحل.