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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ARTICLE INFO

**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 cnemophore 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.,
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 km² 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 (Baioumy 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](image)

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...
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General Authority for Fish Resources Development (GAFRD) semi-annually, particularly mullet fry (El-Serayf *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.](image)

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® 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 ºC with vortexing at 30 min intervals for 5 hours. Samples were then boiled in a 100 ºC dry bath for 20 minutes and then stored at 4 ºC 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 µL, of which 3 µL of total genomic DNA, 0.4 µM of each primer, and 200 ng µL⁻¹ of bovine serum albumin (BSA) were used. The PCR was performed by using 2x DreamTaq™ Green PCR Master Mix (Cat. no. K1081, ThermoFisher) according to the manufacturer’s instructions. The PCR products (3 µL) were electrophoresed in a 2% (w/v) agarose gel, containing 0.5 µg mL⁻¹ 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 brilleae, A. chromis, A. haemuli, A. physoidea, Ceratothoa collaris, C. italica, C. verrucosa, C.terissakai, Cymothoa eremita, C. excisa, C. pulchrum, Elthusa sacciger, E. vulgaris, Ichthyoxenus tanganyikae, Joryma hilsae, Livoneca redmanii, Motocya parvostis, M. collettei, Nerocila longispina, N. phaiopleura, and N. japonica. The primitive free-living isopod Phreatomeres 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 v1.3.1.

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
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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 ischiium. 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.

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

*Note*, values are means ± SD, *n* = 10 males and 1 female. L, length and W, width.
Fig. 3: *L. redmani* 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/cephalan 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 dactyulus. 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                        | COI distance to L. redmanii in Lake Qaroun | COI distance to L. redmanii in the Suez Canal | COI distance to L. redmanii in coastal Egypt | COI distance to L. redmanii in Lake Qaroun | COI distance to L. redmanii in the Suez Canal | COI distance to L. redmanii in coastal Egypt | COI distance to L. redmanii in Lake Qaroun | COI distance to L. redmanii in the Suez Canal | COI distance to L. redmanii in coastal Egypt | COI distance to L. redmanii in Lake Qaroun | COI distance to L. redmanii in the Suez Canal | COI distance to L. redmanii in coastal Egypt | COI distance to L. redmanii in Lake Qaroun | COI distance to L. redmanii in the Suez Canal | COI distance to L. redmanii in coastal Egypt |
|--------------------------------|------------------------------------------|-----------------------------------------------|---------------------------------------------|------------------------------------------|-----------------------------------------------|---------------------------------------------|------------------------------------------|-----------------------------------------------|---------------------------------------------|------------------------------------------|-----------------------------------------------|---------------------------------------------|------------------------------------------|-----------------------------------------------|---------------------------------------------|---------------------------------------------|
| L. redmanii                    | 0.000                                    | 0.000                                         | 0.000                                       | 0.000                                    | 0.000                                         | 0.000                                       | 0.000                                    | 0.000                                         | 0.000                                       | 0.000                                    | 0.000                                         | 0.000                                       | 0.000                                    | 0.000                                         | 0.000                                       | 0.000                                       |
| L. indica                      | 0.000                                    | 0.000                                         | 0.000                                       | 0.000                                    | 0.000                                         | 0.000                                       | 0.000                                    | 0.000                                         | 0.000                                       | 0.000                                    | 0.000                                         | 0.000                                       | 0.000                                    | 0.000                                         | 0.000                                       | 0.000                                       |
| L. exigua                      | 0.000                                    | 0.000                                         | 0.000                                       | 0.000                                    | 0.000                                         | 0.000                                       | 0.000                                    | 0.000                                         | 0.000                                       | 0.000                                    | 0.000                                         | 0.000                                       | 0.000                                    | 0.000                                         | 0.000                                       | 0.000                                       |
| L. thresherorum                | 0.000                                    | 0.000                                         | 0.000                                       | 0.000                                    | 0.000                                         | 0.000                                       | 0.000                                    | 0.000                                         | 0.000                                       | 0.000                                    | 0.000                                         | 0.000                                       | 0.000                                    | 0.000                                         | 0.000                                       | 0.000                                       |
| C. indica                      | 0.000                                    | 0.000                                         | 0.000                                       | 0.000                                    | 0.000                                         | 0.000                                       | 0.000                                    | 0.000                                         | 0.000                                       | 0.000                                    | 0.000                                         | 0.000                                       | 0.000                                    | 0.000                                         | 0.000                                       | 0.000                                       |
| L. praegustator                | 0.000                                    | 0.000                                         | 0.000                                       | 0.000                                    | 0.000                                         | 0.000                                       | 0.000                                    | 0.000                                         | 0.000                                       | 0.000                                    | 0.000                                         | 0.000                                       | 0.000                                    | 0.000                                         | 0.000                                       | 0.000                                       |
| L. praegustator                | 0.000                                    | 0.000                                         | 0.000                                       | 0.000                                    | 0.000                                         | 0.000                                       | 0.000                                    | 0.000                                         | 0.000                                       | 0.000                                    | 0.000                                         | 0.000                                       | 0.000                                    | 0.000                                         | 0.000                                       | 0.000                                       |
| A. physodes                    | 0.000                                    | 0.000                                         | 0.000                                       | 0.000                                    | 0.000                                         | 0.000                                       | 0.000                                    | 0.000                                         | 0.000                                       | 0.000                                    | 0.000                                         | 0.000                                       | 0.000                                    | 0.000                                         | 0.000                                       | 0.000                                       |

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 orbignyi (Abdel-Latif, 2016; Mahmoud et al., 2016; Younes et al., 2016; Shaheen et al., 2017) and Renocila 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 Cymothoae; 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). \(^1\) Livoneca ovalis is recognized as a junior synonym of Livoneca redmanii.

<table>
<thead>
<tr>
<th>Published name</th>
<th>Source</th>
<th>Corrected name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anilocra leptosoma</td>
<td>Rania and Rehab (2015)</td>
<td>Livoneca redmanii Leach, 1818</td>
</tr>
<tr>
<td>Anilocra physodes</td>
<td>Mahmoud et al. (2017)</td>
<td>Anilocra sp.</td>
</tr>
<tr>
<td>Anilocra physodes (^2)</td>
<td>Shaheen et al. (2017)</td>
<td>Immature? Livoneca redmanii Leach, 1818</td>
</tr>
<tr>
<td>Anilocra meridionalis</td>
<td>Youssef et al. (2014)</td>
<td>Anilocra sp. (possibly two species)</td>
</tr>
<tr>
<td>Cymothoa exigua</td>
<td>Youssef et al. (2014)</td>
<td>Cymothoa sp.</td>
</tr>
<tr>
<td>Livoneca redmanii</td>
<td>Helal and Yousef (2018)</td>
<td>Livoneca redmanii Leach, 1818</td>
</tr>
<tr>
<td>Livoneca ovalis(^1)</td>
<td>Shaheen et al. (2017)</td>
<td>Livoneca redmanii Leach, 1818</td>
</tr>
<tr>
<td>Nerocila orbignyi</td>
<td>Abdel-Latif (2016)</td>
<td>Livoneca redmanii Leach, 1818</td>
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<tr>
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<td>Renocila threshorum</td>
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</tr>
<tr>
<td>Renocila threshorum</td>
<td>Ali and Aboyadak (2018)</td>
<td>Livoneca redmanii Leach, 1818</td>
</tr>
<tr>
<td>Isopoda</td>
<td>Elgendy et al. (2017)</td>
<td>Livoneca redmanii Leach, 1818</td>
</tr>
</tbody>
</table>

**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. threshorum (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 antennas/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. threshorum 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. threshorum 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. orbignyi 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 (3rd – 7th) 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 pigmentation patterns on the isopod’s cephalon, 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. orbignyi 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. orbignyi 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;
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 ≥ 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. leidyi*, 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 (~40 °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. leidyi* 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*
Molecular and morphological confirmation of an invasive isopod, *L. redmanii*<sup>sensu lato</sup>, 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 in needed to resolve longstanding taxonomic uncertainties surrounding this species.

**ACKNOWLEDGMENTS**

The authors would like to acknowledge the roles of Amy Driskell and Lee Weigt (Laboratories of Analytical Biology of the National Museum of Natural History, Smithsonian Institution, Washington DC, USA) for sequencing the Chesapeake Bay isopod samples. We are extremely grateful to Niel L. Bruce (Queensland Museum, Australia) for helpful discussions regarding the status of *L. redmanii* and *L. ovalis*, assistance reviewing the literature regarding Mediterranean cymothoids, and reviewing early drafts of this manuscript. We are also grateful to Katrina Lohan (Smithsonian Environmental Research Center) for helpful discussions regarding phylogenetic analyses.

**REFERENCES**


Molecular and morphological confirmation of an invasive isopod, *L. redmanii*

From: "Livoneca redmanii Leach, 1818, 273

**ARABIC SUMMARY**

تأكيدي جزيئي ومورفولوجي لتشابه البرمائي الأمريكي الغازي من منطقة البحر الأبيض المتوسط إلى بحيرة قارون، مصر

خالد محمد جنب، شهير خليف شربة، روبرت أوجير،""

**SUMMARY**

Confirmation of an invasive isopod, *Livoneca redmanii* Leach, 1818, and its distribution from the Mediterranean Sea to Lake Qarun, Egypt.

""""