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The occurrence of new records of Hydroidolinae (Cnidaria: Hydrozoa: Diphyidae) collected from shah bunder creek, Indus deltaic area (Pakistan)

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Abstract
Although Hydroidolinae are abundant and ecologically important in Pakistan, their study appears to be lacking. The genus *Lensia* has 37 species, which are found all over the world. It is the most diverse genus of the Hydroidolinae. Only five species have been recorded in Pakistani waters. However, three species, *Lensia meteori* (Leloup, 1934), *L. hardy* Totton, 1941 and *L. havock* Totton, 1941 are new occurrence of Shah Bunder creek, Indus deltaic area. An identification key and morphological description of these three species are presented in this study.

Keywords: Occurrence, Hydroidolinae, shah bunder, Indus delta (Pakistan)

1. Introduction
There are relatively few types of cnidocysts in the phylum Cnidaria, which is composed of relatively simple aquatic organisms [2]. Cnidaria are classified into five classes: Anthozoa, Cubozoa, Hydrozoa, Scyphozoa and Staurozoa. Hydrozoans exhibit the greatest morphological diversity [2]. Hydroidolina is composed of about 3,350 species, divided into three orders: Anthoathecata, Leptothecata, and Siphonophores [3, 4]. Almost all hydrozoans with a benthic polypoid or hydroid stage belong to Hydrodroidolina (with Limnomedusae being a Trachylina taxon). In Hydroidolinae, colonial hydroid stages, particularly siphonophores, are more specialized than other colonial members of Cnidaria [5-11]. It has been classified into three groups based on their characteristics: Cystonectae, which possess a pneumatophore but no nectophores; Physonectae, which possess both nectophores and pneumatophores; and Calycophorae, which possess nectophores but do not possess pneumatophores [12-14]. Small rocket-like colonies are typical of most siphonophores species, rarely exceeding 20 cm in size [15]. Diphyidae is the family in which most of these species originate [16]. The Siphonophora group, which consists largely of diphyid calycophoran siphonophores, includes *Lensia* Totton, 1932 as one of its most species-rich genera. The genus has cosmopolitan distribution. Despite the poor knowledge, there are few data published about Diphyidae from Pakistani waters from these five are *Lensia* species (*Lensia campanella* (Moser, 1917), *Lensia cossack* Totton, 1941, *Lensia hotspur* Totton, 1941, *Lensia subtilis* (Chun, 1886), *Lensia subtiloides* (Lens and van Riemsdijk, 1908) studied by Ali-Khan and Shehnaz) [17, 18]. This paper discusses the taxonomy of planktonic hydroidolinae species of *Lensia meteori*, L. *hardy* and L. *havock.*

2 Materials and Methods
2.1 Collection, fixation and preservation of sample
The studied specimens were obtained as of Shah Bunder creek (24º 08'39" E 67º 55'18" N) (Figure 1) in 2018. Plankton nets can be used to catch Hydromedusae, which are slowly towed for about 10 - 20 minutes. Hydro-Bios ring trawls of 500 and 250µ mesh size were towed horizontally for ten minutes haul at a constant speed of 0.5 m/s to collect plankton samples at high tide. We used a Hydro-Bios digital flow meter to measure the water flow through the net. As soon as the samples were collected, they were preserved in formalin at 5%. A common taxonomic technique is to fix hydromedusae within seawater by 5% buffered formaldehyde. Fixatives including alcohol ought to be avoiding since they reason contraction, twist, and tightening of the specimens [19].

Physico-chemical parameters as well as water temperature, air temperature, salinity, pH, dissolved oxygen and transparency was documented.

2.2 Microscopic examination

During the laboratory experiment, the samples were divided into aliquots (sub samples). The hydromedusae were isolated from diverse taxonomic groups, counted, photographed, and illustrated with a Leica M3C Stereo microscope and Leica WILD M3C camera (10X/21B, 6.4,10,16,25 and 40 magnifications). After sorting out hydromedusae, they were put into 70% alcohol instead of 5% formalin. To identify the specimens, photographs and illustrations were taken of the best-preserved specimens. Medusae were identified at the highest taxonomic level using their morphology and appropriate taxonomic keys [15, 20, 21].

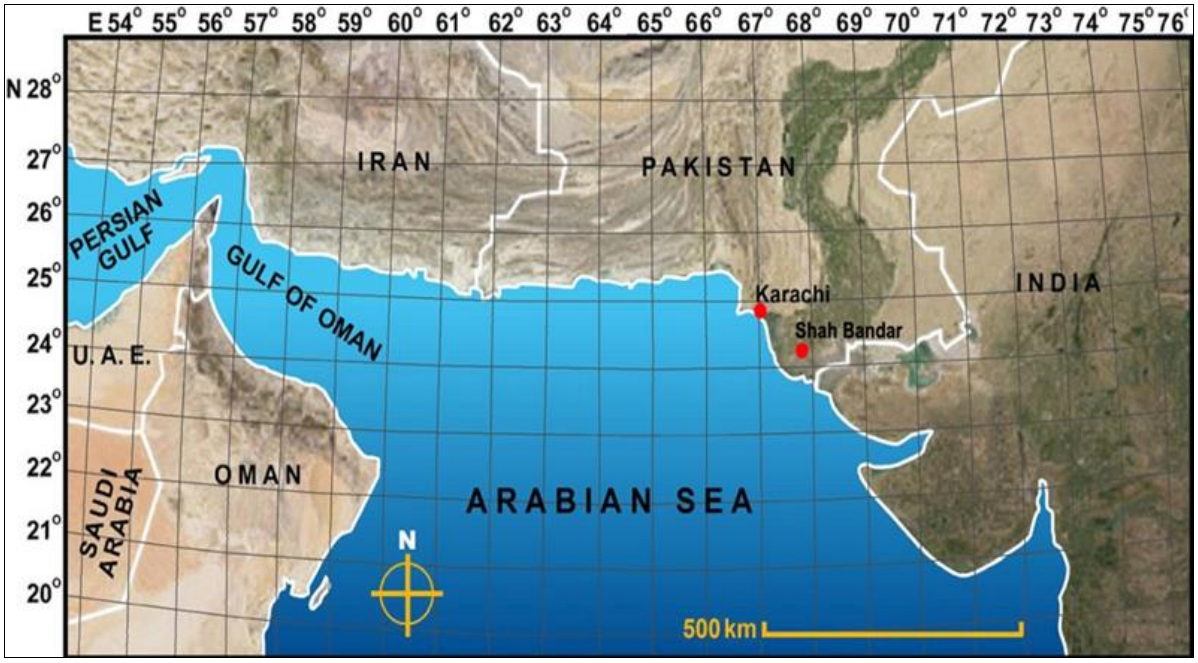


Fig 1: Map showing the collection site

2.3 Measurements (mm) of the *Lensia* species examined. (ANH) anterior nectophores height, (ANW) anterior nectophores width, (NH) necrosis height, (OW) osmium width, (SL) somatocyst length [21].

Table 1: Except for a few specimens that were damaged or poorly preserved

Family / Species	ANH	ANW	NH	OW	SL
Diphyidae					
Lensia meteori	2.1- 2.5	1.11-1.57	1.78-2.61	0.48-0.80	0.33-0.71
Lensia hardy	1.2 - 4.7	1.14 - 2.50	1.81-4.40	0.87-1.63	0.32-0.64
Lensia havock	2.4 - 2.7	1.19 - 2.30	1.88 - 2.27	0.73 - 1.92	0.29 - 0.63

3 Results

3.1 Systematics [22]

Kingdom Animalia
Phylum Cnidaria Hatschek, 1888
Subphylum Medusozoa
Class Hydrozoa Owen, 1843
Subclass Hydroidolinae Marques & Collins, 2004
Order Siphonophores Eschscholtz, 1829
Suborder Calycophorae Leuckart, 1854
Family Diphyidae Quoy & Gaimard, 1827
Subfamily Diphyinae Quoy & Gaimard, 1827
Genus *Lensia* Totton, 1932
Species *Lensia meteori* (Leloup, 1934) (Fig.2)
Species *L. hardy* Totton, 1941 (Fig.3)
Species *L. havock* Totton, 1941 (Fig.4)

3.2 Identification key of *Lensia* species

1. Nonexistence of longitudinal ridges.....2
1'. Existence of longitudinal ridges.....3
2. Somatocyst spherical or laterally expanded on short axis.....*Lensia meteori*
3. Seven longitudinal ridges..... *Lensia havock*
3'. Five longitudinal ridges..... *Lensia hardy*

3.3 Taxonomic descriptions

3.3.1 Hydroidolinae Marques and Collins, 2004

Hydroidolinae are small predatory animals [23] exhibiting radial symmetry. They are characterized by the presence and formation of an exoskeleton [24]. Its chemical composition, rigidity, thickness, and coverage differ from colony to colony during the polypoid stage. Exosarc is derived from epidermal secretions, which are first produced by glandular epidermal cells. Exoskeletons can either be bilayers or contain a perisarc and an exosarc [24].

3.3.2 Diphyidae Quoy and Gaimard, 1827

In general, there are two, sometimes only one, smooth nectophores. Normally, the anterior is pointed apically and located directly above the posterior, which is frequently

truncated apically. In both nectophores, the necrosis occupies a considerable portion of space. Frequently, but not always, the hydroecium of the anterior one is diminished or almost nonexistent. Phyllocysts are usually erratically shaped with helmet-shaped bracts. Except for one or two species, there are no bracteal canals. Whether longitudinal ridges are present or not. [25].

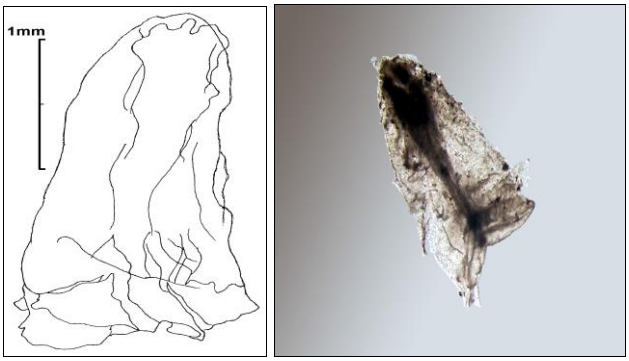
3.3.3 Genus *Lensia* Totton, 1932

The anterior nectophores are generally pyramidal, and have ridges. There are a variety of them and their locations differ. Hydroecium rarely extends above ostial level on the mouth plate. Ostial teeth not present. The mouth plates of posterior nectophores are rounded and truncate apically. Helmet-shaped bracts. In general, anterior nectophore phyllocysts resemble somatocysts. It contains over thirty species that lack the distinctive characteristics of other genera. The number of ridges determines whether a species has 7 or fewer ridges, all running longitudinally, or multiple ridges [25].

3.3.4 *Lensia meteori* (Leloup, 1934) (Fig.2)

Synonyms: *Galetta meteori* Leloup, 1934: 15, fig. 6A-D
Material examined: 27
Diagnostic Features:
Fragile, with rounded apex, anterior nectophores without any obvious longitudinal ridges. A shallow hydroecium extends above oxtail level and is open ventrally on this mouth plate that is narrow, divided into two lappets. Somatocyst have a short stalk and are laterally expanded.

Distribution: Cosmopolitan.
Habitat: An epipelagic species found in the water column.



A) B)

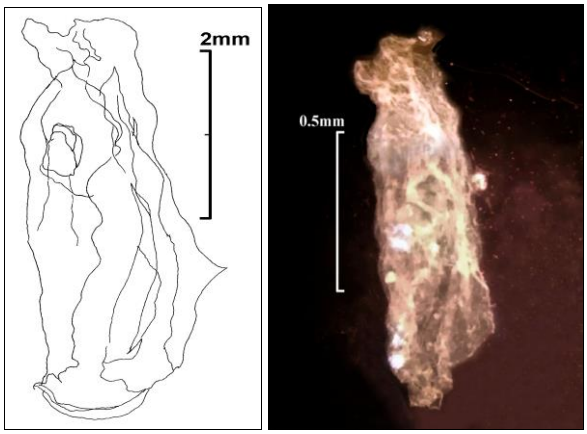
Fig 2: *Lensia meteori* (Leloup, 1934) anterior nectophore seen from the side (A & B)

3.3.5 *Lensia hardy* Totton, 1941 (Fig.3)

Material examined: 19
Diagnostic Features: Nectophores have five longitudinal ridges from tip to ostium. A thin crest covers all the ridges. Below the hydroecium are spherical somatocysts. Medium-sized statocysts are present. The radial canals are joined by circular canals, which follow the typical sigmoid shape. The posterior nectophore has a spherical apex.

Distribution: Cosmopolitan.

Habitat: An epipelagic species found in the water column.



A) B)

Fig 3: *Lensia hardy* Totton, 1941, anterior nectophore seen from the side (A & B)

3.3.6 *Lensia havock* Totton, 1941 (Fig.4)

Synonyms: *Lensia havock* Totton, 1941: 160, figs 17–19; Totton, 1965: 175, fig. 114; Alvari~no, 1981: 438, fig. 174.45; Kirkpatrick and Pugh, 1984: 95, fig. 38; Alvari~no, 1985: 80, fig. 7; Daniel, 1985: 376, fig. 100; Alvari~no *et al*., 1990: 35, fig. 53; Pugh, 1999: 505, fig. 3.93; Gao *et al*., 2002: 130, fig. 70– Illustrations of *L. cordata*: 131, fig. 71 and *L. havock* inverted); Mapstone, 2009: 192, fig. 49. *Muggiaea havock* Stepanjants, 1967: 185, fig. 126; Zhang, 2005: 65, fig [15].

Material examined: 34
Diagnostic Features: In the anterior nectophore, there are seven longitudinal ridges, with upper laterals curving upwards to reach the ostial level, and lower laterals curving downwards to form the mouthplate. Hydroeciums extend anteriorly of the ostial level, with a triangular posterior notch; somatocysts are club-shaped with pedicles. There are teeth-like projections on the basolateral corners of the neck shield of phyllocysts; conical bracket; headpiece without distinct ridges. The gonophore has six ridges with only two extending into the large, rounded mouthplate; the hydroecium is shallow; the articulate surface is concave; and the nectosac is broad, with a rounded, conical tip.

Distribution: Cosmopolitan.

Habitat: An epipelagic species found in the water column.

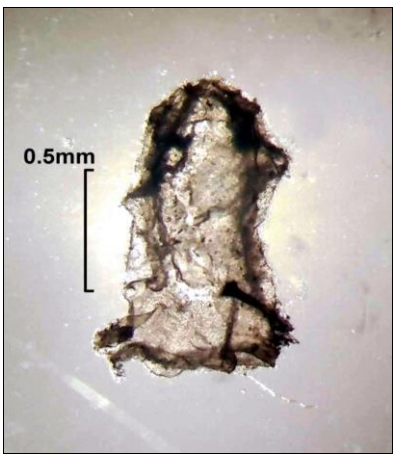


Fig 4: *Lensia havock* Totton, 1941, anterior nectophore seen from the side

4. Discussion

In the Indus deltaic area of Pakistan, we studied three unrecorded *Lensia* species found along the Shah Bunder creek. Five species have previously been recorded, *Lensia campanella* (Moser, 1917), *Lensia cossack* Totton, 1941, *Lensia hotspur* Totton, 1941, *Lensia subtilis* (Chun, 1886), *Lensia subtiloides* (Lens and van Riemsdijk, 1908) from northern Arabian sea [17, 18].

Marine plankton is dominated by siphonophora, which occur abundantly in tropical seas. Due to their fragility and location in deep waters, most of these animals are poorly studied because they can't be collected or preserved [8]. Calycophore siphonophorans of the Diphyidae family have an anterior nectophore with a distinctive bullet shape that makes them morphologically unique. Despite this, the species of Diphyidae have many similarities morphologically, making it difficult to differentiate them by species or genera. At present, there are 26 species in the *Lensia* genus, which is by far the largest within the family [15]. Due to their high water content, gelatinous Diphyids are problematic for taxonomic analysis since they are dehydrated when fixed in ethanol. The shriveled body shape increases the chances of misclassification since morphological features are difficult to identify. As a result of folds caused by dehydration, the longitudinal ridge boundaries of many species became unclear [26].

In total, 92 specimens of *Lensia* were analyzed, with three new species being found in Pakistani waters. Measurements of examined specimens are given in Table 1. Except for a few specimens that were damaged or poorly preserved, the morphological descriptions were consistent with the literature. There were some structures that were difficult to differentiate and could not be measured or described in those cases. Some species could be easily identified because of the number and shape of longitudinal ridges on the somatocyst, and the relative positions of the hydroecium and somatocyst on the osmium. The species are described using the terminology by Haddock *et al*., [21, 27, 28]. Diphyidae are Holoplankton in habit [29], and their ability to adapt themselves to varying hydrographic conditions means that their presence in Pakistani waters is anticipated.

5. Conclusions

Despite its abundance, gelatinous zooplankton still needs proper management as they provide a habitat for various marine mammals [30, 31]. An evaluation of hydrozoa from our waters would be a pioneering effort, as there is virtually no knowledge of them. By improving our systematic familiarity with the Indus deltaic hydrozoan fauna, the present study contributes to our understanding of this ancient ecosystem.

6. Acknowledgements

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