

NEW RECORDS AND MOLECULAR IDENTIFICATION OF *LEPTOPLANA TREMELLARIS* (MÜLLER OF, 1773) ORSTED, 1843 (PLATYHELMINTHES, RHABDITOPHORA, POLYCLADIDA) FROM THE BULAJI ROCKY SHORE COASTAL WATERS OF PAKISTAN

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ABSTRACT

The present study reports the new records and molecular identification of *Leptoplana tremellaris* (Müller OF, 1773) Orsted, (1843) (Platyhelminthes, Rhabditophora, Polycladida), from Pakistan. Previously, ten species of Polycladida representatives of the family Pseudocerotidae recorded from Pakistan. In the current study the representatives of the family Leptoplanidae (Polycladida) *L. tremellaris* collected from the Bulaji rocky shore (N 24° 51' 571" E 66° 52' 157") coastal waters of Pakistan and confirm the occurrence of species based on morphological and molecular characteristics.

Keywords: Polycladida, Leptoplanidae, *Leptoplana tremellaris*, new record, Pakistan.

INTRODUCTION

Turbellarians are abundant species found in marine and brackish waters (Faubel and Noreña, 2001) the initial review of Turbellaria of the world was described by (von Graff, 1904-08). Recent accounts on marine Polycladida, and Turbellaria were published by Bock (1913) and an overview of the Turbellaria was described by (Faubel, 1983, 1984 and Prudhoe, 1985). According to the new phylogenetic system, after the evaluation of renowned polyclads, of the order, Polycladida was subdivided into two suborders: Acotylea and Cotylea. *Leptoplana tremellaris* (Marine flatworms) type species of the genus *Leptoplana* eminent polyclad belong to the family Leptoplanidae, has a worldwide distribution and re-described by Bock (1913) and Faubel (1983). It found in marine sediments and common in the Atlantic coast of Europe, and intertidal areas of Great Britain and Ireland.

Recently *L. tremellaris* was placed within the family Leptoplanidae Stimpson (1857), Prudhoe (1985) accepted its taxonomic position within the subfamily Leptoplaninae Marcus (1947) of the family Leptoplanidae. Despite the economic importance of acotylean flatworms as pests, they have received very little taxonomic attention. Gammoudi *et al.*, (2009) describe Acotylean Polyclads (Platyhelminthes Polycladida) from Tunisian Coasts. Gammoudi *et al.*, (2012b) thoroughly re-describe Polycladida from the Mediterranean Sea and elevate the two sub-species of genus *Leptoplana*: *L. tremellaris* and *L. mediterranea* Bock, (1913) based on the specific external morphological characters. Tyler, (2014) reported *L. tremellaris* as a European registered fauna of marines, Pawar and Al-Tawaha (2017) reported *L. tremellaris* from the Uran coast, Navi Mumbai, Arabian Sea. Gammoudi *et al.*, (2017) again resolved controversial issues and reported the contentious appearance of the *L. tremellaris* in the Mediterranean Sea and supported it with detailed embryological and histological evidence.

Kazmi, (1996) described some polycladids from the coastal waters of Pakistan, while Kazmi and Naushaba (2013) published a checklist of marine worms reported from Pakistani marine waters including the genus *Pseudoceros susanae*, *Tytthosoceroslizardensis* and *Cestoplanarubrocinta*. Kazmi (2016) also reports ten new records of polycladids from Pakistani marine fauna belonging to the family Pseudocerotidae (Lang 1884). During the present study, *L. tremellaris* was reported as a new record from the rocky shore of Buleji the coastal waters of Pakistan.

MATERIAL AND METHODS

The rocky ledge at Buleji (N 24° 51' 571" E 66° 52' 157") is a gradually sloping, triangular stretch, of a rocky raised strip which protrudes out into the Arabian Sea (Fig. 1). Buleji is an exposed wave trampled shore with slightly elevated and depressed areas. This area is a composite of sand and gravel, cobbles and pebbles with a rocky base. The ledge is particularly rich in invertebrate fauna. The specimen of *L. tremellaris* was collected from Buleji rocky

shore coastal waters of Pakistan and immediately transferred into a plastic bottle filled with seawater before, later examination in the laboratory. Taxonomic identification of *L. tremellaris* was based on Gammoudi *et al.*, (2009), Marquin *et al.* (2015), Gammoudi *et al.* (2017). In the laboratory, specimens were measured and photographed using an SZ61 Zoom Stereomicroscope and DP22 colour camera. Morphometric measurements were observed to the nearest 0.1 mm.



Fig. 1. Map of the study area Buleji from the coastal waters of Pakistan.

Molecular Identification

DNA extraction and sequence analysis

For molecular identification mussel tissues are used for the extraction of the genomic DNA (gDNA); the process was done by Qiagen's DNeasy blood and tissue kit cat. No. 69504. A 694-base pair (bp) of the target DNA segments were amplified by the polymerase chain reaction using two universal primers of the COI gene; LCO1490: 5'-ggtaacaaatcataaagatattgg-3' and jg HC02198: 5'-taaacttcagggtgaccaaataatca-3' (Folmer *et al.*, 1994) in the mt-DNA genome. The Polymerase Chain Reaction was completed in an applied Bio-system 2720 thermal cyclor. The thermal cycling profile was carried out with the following steps: denaturation (initial) cycle of 10 min at 95°C followed by 40 cycles of 1 min at 95°C 1.5 min at 50°C and 1 min at 72°C with a final extension of 72°C for 10 min. A new sequence of data was submitted to the GenBank. DNA sequences were examined for homology-based on blasting 2.2.26+ (Zhang *et al.*, 2000). All the DNA sequences were initially aligned using Clustal W Thompson *et al.* (1994) evolutionary relationship was analyzed by the maximum likelihood method according to the Tamura-Nei model (Tamura and Nei, 1993) and the genetic heterogeneity within species of *L. tremellaris* was estimated by using MEGA 7 (Kumar *et al.*, 2016).

Systematic accounts

Phylum Platyhelminthes

Class Rhabditophora

Sub class Trepaxonemata
Order Polycladida
Sub order Acotylea Lang 1884
Superfamily Leptoplanoidea Faubel 1984
Syn.: Schematommatidea Böck 1913
Family Leptoplanidae Stimpson 1857
Genus *Leptoplana* Ehrenberg, 1831
Species *Leptoplana tremellaris* (Müller 1773) Oersted 1843

Synonyms

Fasciola tremellaris Müller, 1774
Planaria flexilis Dalyell, 1814
Leptoplana flexilis Lankester, 1866

Type material

Leptoplana tremellaris three specimen

Taxonomic History

Leptoplana tremellaris Fig. 4 (A-F) and 5 (A-C), Gammoudi *et al.* (2009): Fig. 5. (A) p. 9 Marquina *et al.* (2015): (Fig. 2 b, Fig. 3 a–I), Gammoudi *et al.* (2017).

Type locality

Buleji rocky shore (N 24° 51' 571" E 66° 52' 157") Karachi, Pakistan.

Diagnosis

Delicate, deltoid, oval-shaped elongated body along with wavy margins, light ash brown color, luminous, with dorsal brownish shade (Fig. 2A, 2C, 2D). Tentacles absent, tentacular eyes arranged in two rounded clusters. Cerebral eyes arranged in two elongated patches, on either side of the tentacular eye clusters (Fig. 2B) to the reproductive organs, reproductive organ in the second half of the body, darker than body pigmentation. Testes found in ventral position ovaries found in the dorsal position. Copulatory organ complex in male with interpolated prostatic vesicle. Penis papilla without stylet or cirrus. Female system with the typical truncated S-shape, with few muscles and abundant cement glands. A characteristic genital sucker is located between the male and female organs.

Identification

Identification based on the external and internal morphological characters based on (Gammoudi *et al.*, 2009, Marquin *et al.*, 2015, Gammoudi *et al.*, 2017)

The coloration of fresh specimen

Light ash brown

Biology

Intertidal region or littoral zone under stones, among sand and gravel under boulders

Size

Length 25 mm, Width 10 mm

Distribution

Isles of Scilly (Faubel and Warwick (2005), Cantabrian coast (North Atlantic) Iberian Peninsula Marquina *et al.* (2015), Pawar and Al-Tawaha (2017) Uran coast, Navi Mumbai, Arabian Sea, Tunisian Coasts Northern Africa, bordering the Mediterranean Sea, current study Buleji cost Karachi Pakistan.

Conservation Status according to IUCN

Very common

Specimens Deposition

Leptoplana tremellaris three specimen deposit in the Marine Reference Collection and Resource Centre University of Karachi.

Remarks

L. tremellaris was identified as a new record from the coastal waters of Pakistan based on morphological and morphometric characters. Previously *L. tremellaris* was reported from the Isles of Scilly, the Cantabrian coast (North Atlantic) Iberian Peninsula, Uran coast, Navi Mumbai, the Arabian Sea and Tunisian Coasts of Northern Africa, bordering the Mediterranean Sea. The genus *Leptoplana* was revised by Gammoudi *et al.* (2012b) and confirmed the occurrence *L. mediterranea* in Mediterranean mean while *L. tremellaris* considered a missing species in the area. Gammoudi *et al.* (2017) again resolve conflict issues and the contentious appearance of the *L. tremellaris* in the Mediterranean Sea.

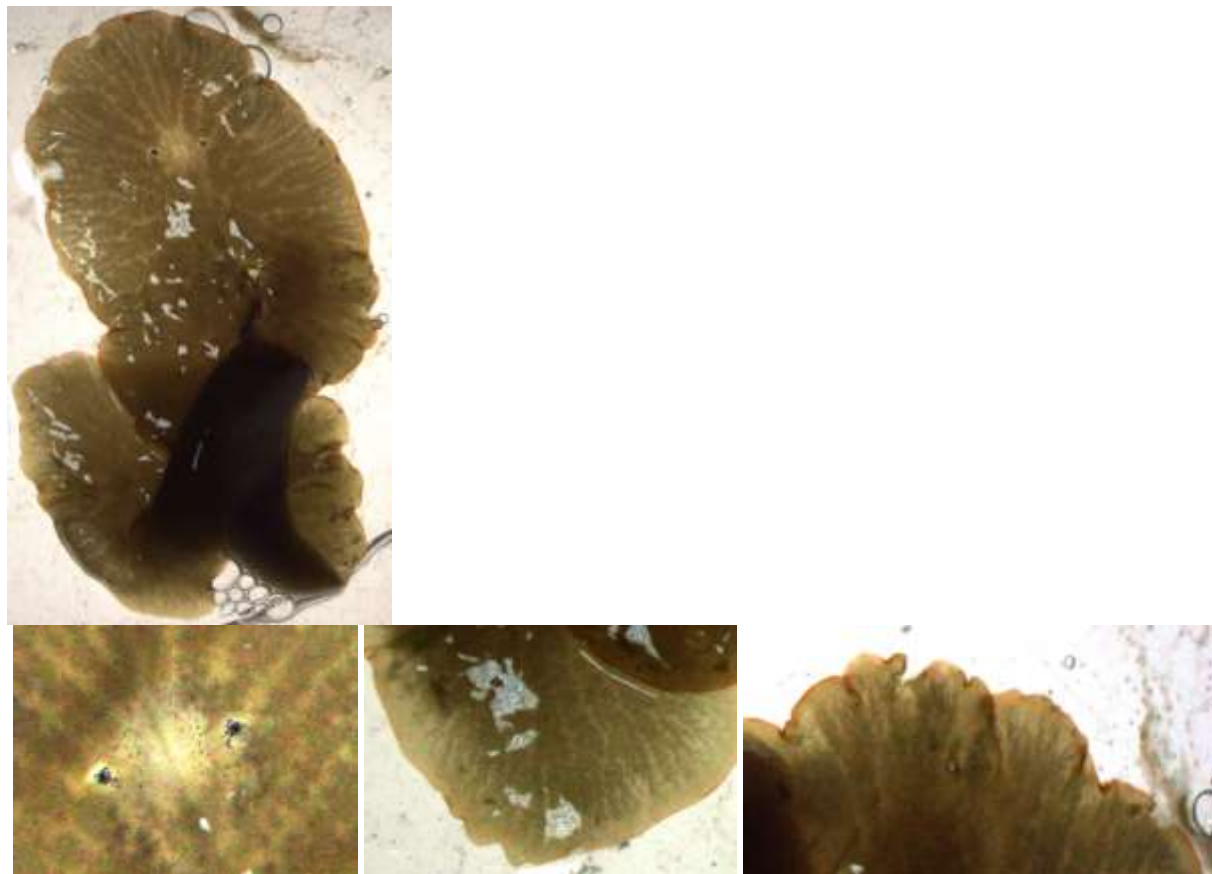


Fig. 2. *Leptoplana tremellaris*, dorsal view of the movable specimen (A) cerebral and tentacular eyes (B) tail area (C) wavy margin during movement (D).

Molecular Remarks

In the present study the molecular identification of *L. tremellaris* based on the coding gene of mt DNA Cytochrome Oxidase 1(COI), the genetic similarity searched through BLASTn. The specimens (n=3) were collected from Buleji rocky ledge Karachi Pakistan by direct hand pick. After amplification, the COI gene exhibits a band presence of UV light, whereas 100 base pair plus DNA ladder Gene Ruler used for the comparison. Sequence similarity searched by using the Basic Local Alignment Search Tool (BLAST), species homology based on at least a 97-100% homology. DNA sequences submitted to GenBank and the accession number received for each isolate (MN396901.1, MN396902.1, MN396903.1) whereas the other previously submitted sequences (MK702075.1, MK713896.1) of *L. tremellaris* was also included in the analysis. The estimation of average evolutionary divergence over all sequence pairs was observed and the number of base substitutions per site from averaging over all sequence pairs was 0.242 analyses were conducted using the Tajima-Nei model 1994. The evolutionary history inferred by using the maximum-likelihood method based on the Tamura-Nei model (Tamura and Nei (1993) (Table 1) over sequence pairs within *L. tremellaris* range between 0.000-0.006. A total of five sequences were used for the phylogenetic analysis and it revealed that the phylogenetic relationship was observed within the representatives of *L. tremellaris* (Fig. 3).

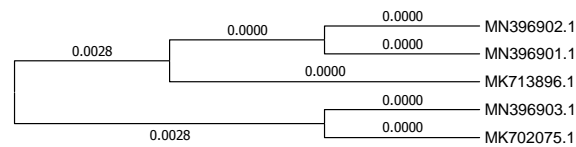


Fig. 3. The phylogenetic relationship between species of *Leptoplana tremellaris* by using the maximum-likelihood method based on the Tamura-Nei model (Tamura and Nei, 1993).

Table 1. Estimation of evolutionary divergence between sequences pair of *L. tremellaris* by using the Tajima-Nei model.

	MN396903.1	MK702075.1	MK713896.1	MN396902.1	MN396901.1
MN396903.1		0.000	0.003	0.003	0.003
MK702075.1	0.000		0.003	0.003	0.003
MK713896.1	0.006	0.006		0.000	0.000
MN396902.1	0.006	0.006	0.000		0.000
MN396901.1	0.006	0.006	0.000	0.000	

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