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First record of *Limnodrilus claparedeianus* Ratzel, 1868 (Annelida: Oligochaeta: Tubificidae) from Anzali Wetland, Guilan province, Iran

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ABSTRACT

Oligochaeta worms are important organisms in aquatic ecosystem. Their omnipresence makes them as an indicator of environmental changes and health of aquatic ecosystem. The members of the family Tubificidae and in particular, genus *Limnodrilus* are considered as the most tolerant oligochaets to aquatic pollution. Therefore identification and biological characteristics of this taxon are of great help to evaluate the ecosystem of water bodies. *Limnodrilus claparedeianus* were collected from Anzali Wetland with a grab of 0.04 m^2 capacity at 13 stations from August 2012 through June 2013. Penis sheath was one of the main characteristics to identify them. Results showed their maximum and the minimum density in spring (601.27 ± 466.8346 $Ind.m^{-2}$) and in autumn (156.12 ± 136.3 $Ind.m^{-2}$) respectively which was significantly different ($P<0.05$). Comparative spatial distribution of *L. claparedeianus* among 13 stations revealed significant differences in the west stations ($P<0.05$). According to the results, correlation analysis did not exhibit any relation between abundance of *L. claparedeianus* and total organic matter, abundance of the species and percentage of silt in substrate sediment.

Key words: Anzali Wetland, *Limnodrilus claparedeianus*, new record.

INTRODUCTION

Benthic macro-invertebrate communities in wetlands are important component of the aquatic environment which plays momentous roles in the energy pathway and nutrient cycling, assessment of the ecological entirety and bio-monitoring of aquatic habitats (Lae, 1994; Gordon, 2000; Acharyya & Mitsch, 2001; MRC, 2010). They also establish an important connection as food reservoir for fishes and other animals in aquatic food chain (Blay & Dongdem, 1996; Arslan *et al.,* 2007). Among benthic communities, oligochaetes are common and abundant animal groups, which are strongly influenced by environmental conditions. Out of more than 5000 known species of class Oligochaeta, approximately 1100 species live in freshwater (Suriani *et al.,* 2007). Environmental richness of oligochaete species is directly related to food availability and quality, substrate type, oxygen availability and biological interactions. Furthermore this group are not much motile and mostly are considered as indicators of specific trophic state of aquatic habitat (Behrend *et al.,* 2012). Generally, oligochaetes have wide geographical distribution patterns and their population can sometimes reach quite large number. Many species prefer eutrophic water, living on muddy sediments with abundant detritus organic matter. Anzali Wetland is an internationally known inland water body in north of Iran and has gained more reputations as being connected to Caspian Sea. It hosts a wide variety of animal species and therefore possesses great ecological concern to remain under continuous monitoring. The present study introduces *Limnodrilus claparedeianus* and its distributional peculiarities as a monitoring index.

MATERIALS AND METHODS

Study area

Anzali Wetland (37° 28′ N, 49° 25′ W) represents an internationally important wildlife reservoir and sanctuary with an open surface area of 58 km² which is listed under Ramsar Convention (Pourang, *et al.* 2010) (Fig. 1). Urbanization and population growth, industrial and agricultural activities, and also tourism are major sources of pollutants discharged into the Wetland directly or via rivers indirectly.

The southern part of the wetland is mainly under rice cultivation and patches of woodland, while the northern part is bordered by sand dunes, grassland and scrubby vegetation (Ayati, 2003).

Sampling and sample preparation

The specimens of *Limnodrilus claparedeianus* were collected seasonally from 13 stations in Anzali Wetland from August 2012 through June 2013 (Fig. 1 and Table. 1). The oligochaetes were collected with grab (0.04 m²) accompanied with bryophytes and other submerged plants and also from bottom surface layer.

The sediments were passed through a 500 µm mesh size sieves on spot and sieved particles were preserved in 10% formalin. In the laboratory, samples were then isolated and transferred to 70% ethanol.

Worm specimens were dipped in Amman's lactophenol for transparency, mounted on microscope slides covered by a cover slip and left for several hours before examination.

All measurements and photographs for preserved specimens were taken and analyzed by TSVIEW 5 mega pixel camera and software (version 6.2.4.5).

Identifications followed detailed description made by Kathman and Brinkhurst (1998) and Douglas (2001).

Certain collected specimens during this study are now deposited in Dr. Tarmo Timm's fresh water annelid collections, Centre for Limnology, Institute of Agricultural and Environmental Sciences, Estonian University of Life Sciences under code number S-4333.

Total organic matter of sediment and the granulometric analysis followed the procedures described by Moghaddasi *et al.* (2009).

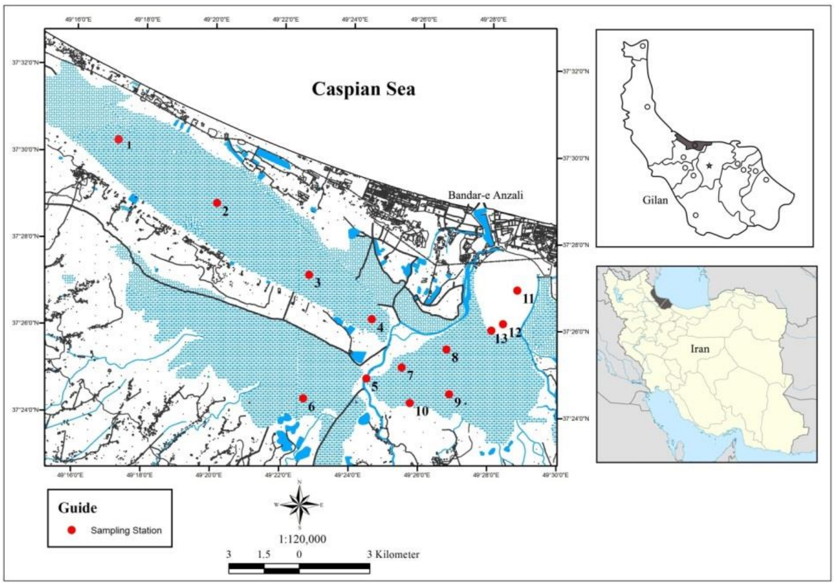

Fig. 1. Study area and sampling sites.

Table 1. Coordination of sampling sites.

Station	Longitude	Latitude
1	37 28 22	49 19 20
2	37 28 50	49 20 06
3	37 27 13	49 22 48
4	37 26 13	49 24 38
5	37 24 51	49 24 30
6	37 24 22	49 22 41
7	37 25 07	49 25 31
8	37 25 33	49 26 48
9	37 24 31	49 26 54
10	37 24 18	49 25 46
11	37 26 56	49 28 49
12	37 26 09	49 28 25
13	37 26 00	49 28 05

Data analysis

Data analysis was done by SPSS version 18. Data normality was assessed using the Kolmogorov-Smirnov test.

For normally distributed data, One-Way ANOVA was performed. Otherwise, the Mann-Whitney U test and Kruskal -Wallis test was applied. For entry data to computer and calculation of mean, standard error, charting and other operations, Excel 2007 was used.

RESULTS

Morphological descriptions

Live specimens were red in color but formaldehyde preserved ones turned into milky cream. Worm body was 7 to 35 mm in length and 3 to 4 mm in diameter. The cylindrical body showed bilateral symmetry and at least 40 to more than 100 segments were counted. Eye spots were absent. Caudal region was undifferentiated with conical prostomium (Fig. 2a, b). Dorsal and ventral chaetae commenced from second body segment, regularly and alternatively at upper and lower regions of body (Fig. 2d). All chaetae were bifid and similar, upper tooth longer than lower tooth or slightly equal and hair were absent in dorsal bundle (Fig. 2c). Anterior segments were comprised of 6-8 chaetae per dorsal bundle and 2 chaetae per ventral bundle. Clitellum was seen in segments X to XII. Like other oligochaets, *L. claparedeianus* was hermaphrodite where male and female organs appeared simultaneously over transparent body wall (Fig. 2a). Paired penial sheath distinguishable in segments XI was one of the main identifying characteristics (Fig. 2: a, g).

Table 2. Abundance (mean ± SD) of *L. claparedeianus*.

season station	Summer	Autumn	Winter	Spring
S1	___	____	25.66 ± 19.68	53.33 ± 18.95
S2	50 ± 44.72	____	_____	124 ± 25.5
S3	86 ± 13.50	____	16.66 ± 12.9	____
S4	236 ± 40.70	246.33 ± 78.34	1485 ± 207.81	53.28 ± 8.68
S5	1139 ± 283.84	226.67 ± 53.23	658.37 ± 66.59	862.66 ± 123.35
S6	581.66 ± 217.36	193 ± 51.71	138.23 ± 56.14	1296.66 ± 292.51
S7	536.33 ± 177.02	96.66 ± 34.97	1303 ± 428.02	456 ± 126.06
S8	3062.33 ± 821.36	358 ± 65.56	217.16 ± 59.3	991 ± 271.91
S9	317.68 ± 54.08	154 ± 69.83	717 ± 267.5	925 ± 530.915
S10	522.66 ± 279.86	456 ± 192.35	266.66 ± 71.39	403 ± 193.19
S11	158.24 ± 87.91	105.36 ± 52.49	240 ± 60.42	484.66 ± 29.62
S12	239 ± 88.32	76.66 ± 32.67	1216.33 ± 394.85	1389 ± 151.84
S13	623.66 ± 234.78	117 ± 28.41	1068.25 ± 317.39	778 ± 153.74

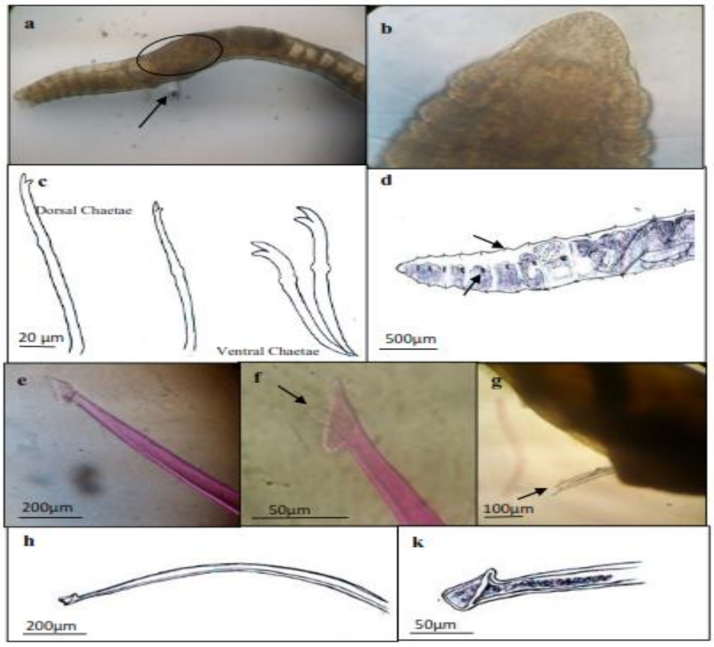

Fig. 2. (a) Anterior end of *L. claparedeianus*, location of penial sheaths (arrow) and clitellum (ellipse). (b) Conical head region of *L. claparedeianus*. (c) Dorsal and ventral chaetae. (D) Location of dorsal (upper arrow) and ventral chaetae (lower arrow). (E, g) micrographs and (h, k) Schematic figures of penial sheath anterior end. (f) Penis (arrow) located within the transparent penial sheath.

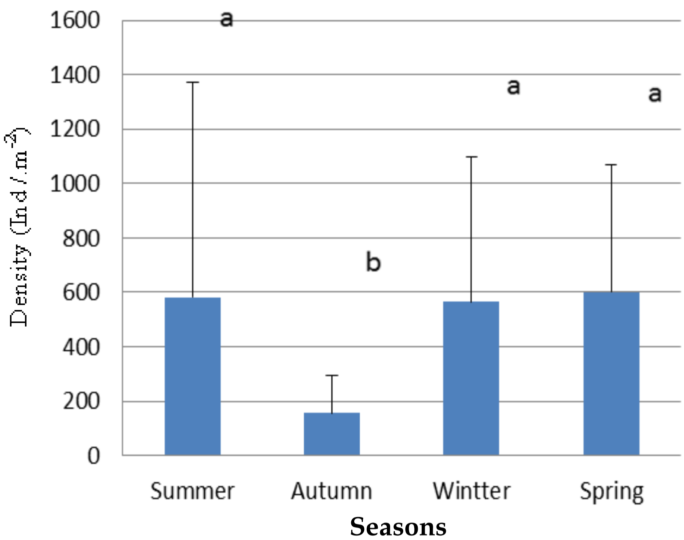

Fig. 3. Seasonal mean abundance of *L. claparedeianus.*

Table 3. List of identified oligochaetes in Anzali Wetland (JICA, 2005).

Family: Naididae	**Family: Lumbriculidae**	**Family: Tubificidae**	**references**
-	-	*Tubifex* sp.	Mirzaee Bafti,1991
-	-	*Tubifex* sp.	Abdolmaleki, 1993
-	*Vareigatus* sp.	*Tubifex* sp.	Khodaparast, 1996
-	-	Tubificidae	Mirzajani *et al*. 1999
Naididae	-	Tubificidae	Ahmadi and Khara, 2006
Naididae	Lumbriculidae	Tubificidae	Mirzajani *et al*. 2009
Nais sp.	*Lumbriculus* sp.	*Tubifex* sp.	JICA, 2005
-	*Lumbriculus* sp.	*Tubifex* sp.	Jalili *et al*. 2011

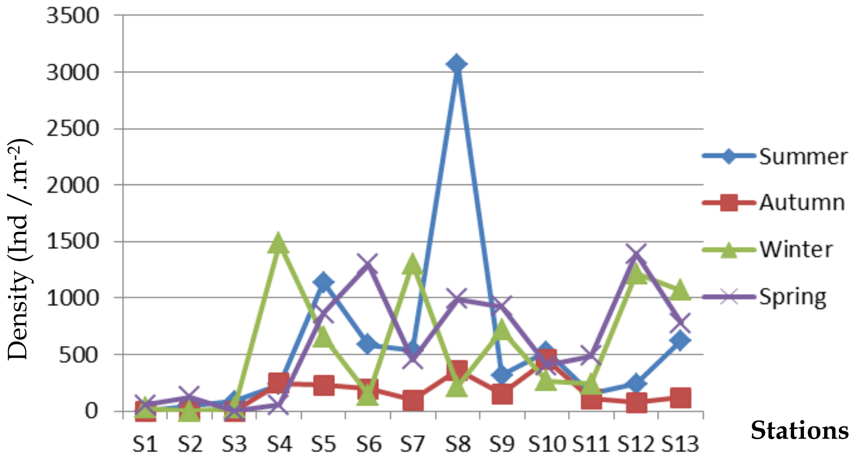

Fig. 4. Mean abundance of *L. claparedeianus* in 13 stations is compared in four seasons.

Spatial and seasonal distribution patterns of *L. claparedeianus*

This species was observed in all seasons and within all mesohabitats. Statistical analysis was performed in order to determine the probability existence of spatial and seasonal patterns relating the abundance to certain physico-chemical parameters. A distinct seasonal maximal mean for abundance *L. claparedeianus* was observed in spring (601.27 ± 466.83 Ind.m^{-2}) and minimum in autumn (156.12 ± 136.3 Ind.m^{-2}). From spatial point of view the maximum abundance mean was observed in station 8 (e.g. Sorkhankol wild life refuge, 1157.12 ± 1216.49 Ind.m^{-2}) and a minimum in western station 1 (19.74 ± 23.55 Ind. m^{-2}) (table1). Seasonal density average was s comparatively significantly different only in autumn ($P<0.05$) (Fig. 3). Spatial distribution of *L. claparedeianus* in stations were compared using Kruskal-Wallis and Mann-Whitney U test, and lack of normal distribution of data was assessed by Kolmogorov-Smirnov test, revealing that the mean abundance of total population of the species among 13 stations was significantly different between western stations and other stations located at Siahkeshim (Southern part), Sorkhankol (central part) and eastern part of the Wetland, ($P<0.05$). Meanwhile, there was no significant difference among different stations of each part. According to the results obtained, correlation analysis did not detect any relations between abundance of *L. claparedeianus* and total organic matter, also between abundance of the species and percentage of silt in substrate sediment.

DISCUSSION AND CONCLUSION

During one year sampling in Anzali Wetland, *L. claparedeianus* was observed for the first time in Iran. Lack of eyes, terminal anus, undifferentiated caudal region, conical prostomium were clear characteristics to identify the Family. However some characters like the number of chaetae per bundle, the shape of the chaetae, availability, length and shape of penis sheath were also used. The upper tooth of chaetae was often longer than the lower one, but these chaetae in some specimens were difficult to distinguish from those of *L. hoffmeisteri* because the upper tooth was only slightly longer than the lower one. The most trusted reliable key to identify *Limnodrilus* genus at species level was the shape of penis sheath particularly the shape of distal end (Kennedy, 1969; Brinkhurst, 1971; Bird & Ladle, 1981; Ohtaka, 1985; Yasuda & Okino, 1987; Pinder & Brinkhurst, 1994; Swayne *et al.*, 2004; Krieger & Stearns, 2010). It is worth noting that variations in the lengths of the oligochaete body, chaetae and the number of segments are common and suspected to be due to locality differences (Al-Abbad, 2012).

Harber (1938) and Sperber (1948) emphasized that these variations in length and number of segments occurred even within the same species.

In a general study (Table 2) 5 reported taxa represented five genera in three families in Anzali Wetland (JICA, 2005) and *L. claparedeianus* was not in the list. Constant observation of this species in all seasons and within all mesohabitats and different substrate of wetland can reveal the fact that the species is compatible to harsh environmental condition and various habitats. Maximum seasonal abundance mean of the *L. claparedeianus* in spring (601.27 ± 466.83 Ind. m^{-2}) while minimum in autumn (156.12 ± 136.3 Ind.m^{-2}) and nonexistent significant difference among other seasons could be due to optimum environmental condition (such as food availability and temperature) in spring. Stephan and Alves (2001) concluded that the high densities of *Limnodrilus* may suggest high amount of food availability (organic matter) together with rare opportunities for biotic interactions such as predation and competition. They are a consequence of water pollution which may play an important role in the fitness of such genus in Pedro stream. Studies on oligochaete species in the Scandinavian lakes based on the degree of enrichment indicated that *L. claparedeianus* belonged to a group of species tolerating extreme enrichment of organic pollution (Rodriguez & Reynoldson, 2011). On the other hand, research works have proven in recent years that water eutrophication has turned to be a serious environmental challenge to Anzali Wetland ecosystem as increasing waste loads from industrial, agricultural and human activities to international has reached an alarming level (Akbarzadeh *et al*., 2008; Mirzajani *et al*., 2010). Since members of the genus *Limnodrilus* are considered as one the most tolerant oligochaetes especially to organic pollutions (Brinkhurst, 1980; Milbrink, 1980; Lang, 1984; Seys *et al*., 1999), their continuous presence in Anzali Wetland might indicate existence of high degrees of organic pollution. It should be noted that classification of some aquatic oligochaetes like *L. claparedeianus* from the St. Lawrence Great lakes has placed the lake in eutrophic level (Rodriguez & Reynoldson 2011).

It can therefore be concluded that densely presence of *L. claparedeianus* in Anzali Wetland in all sampling sites and all seasons except autumn, illustrates high organic pollution load in all parts of the Wetland and also in most seasons.

The effect of organic pollutions could also be observed on wetland regional basis. For example, station 8 (Sorkhankol wild life refuge) located at the south of Anzali Wetland (1157.12 ± 1216.49 Ind.m^{-2}) which provided the maximum *L. claparedeianus* density used to receive large amount of agricultural, urban wastewater, remains of plants and animals, while the least abundance of *L. claparedeianus,* was observed at stations located in the western part of the Wetland.

The western region met lower industrial and agricultural activities and therefore establishing a healthier environmental condition. Anzali Wetland is a complex costal wetland and affected by many environmental parameters.

Suriani *et al.* (2007) stated the species composition, abundance and distribution of benthic invertebrates usually depended on many variables as important as the substrate type, dissolved oxygen concentration, water level fluctuation, biotic food quality and availability, competition and finally predation. In the present study, a correlation between abundance of *L. claparedeianus* and total organic matter and percentage of silt in substrate sediment was not observed.

Yap *et al.* (2006) suggested non-existing correlation between abundance of *Limnodrilus* and total organic matter could indicate that this group is resistant to pollution, however detailed study and discussion on how abiotic factors influence the distribution of the resistant *Limnodrilus* sp. has not yet been discussed in the literature. According to the results obtained through the present study it could be concluded that permanent presence of *L. claparedeianus* in highly TOM loaded (19.56 ± 7.04), Anzali Wetland is a warning sign which has to be considered seriously.

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نخستین گزارش گونه *Limnodrilus claparedeianus* **Ratzel, 1868** (کرم حلقوی، کم تار، توبیفیسیده) از تالاب انزلی، استان گیلان، ایران

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چکیده

کرمهای کم تار از اجزای مهم اغلب اکوسیستم‌های آبی هستند. حضور فرا گیر این گروه جانوری سبب شده تا به عنوان شاخص تغییرات زیست محیطی و سلامت محیط های آبی مورد استفاده قرار گیرند. اعضای خانواده توبیفیسیده و بویژه جنس لیمنودریلوس از کم‌تاران مقاوم نسبت به آلودگی محیط آبی هستند. از این رو شناسایی و بررسی خصوصیات بیولوژیک این گروه کمک مهمی به ارزیابی کیفیت پیکره‌های آبی خواهد کرد. برای این منظور *Limnodrilus claparedeianus* با استفاده از گرب با سطح ۰/۰۴ مترمربع و در ۱۳ ایستگاه تالاب انزلی ار تابستان۹۱ تا بهار ۹۲ جمع‌آوری گردید. غلاف اندام تناسلی نر یکی از خصوصیات اصلی در تشخیص و شناسایی این کرمها بود. نتایج نشان داد که بیشترین تراکم فصلی این گونه کم تار در بهار (۴۶۶/۸۳ ± ۶۰۱/۲۷ عدد در مترمربع) و کمترین آن در پاییز (۱۳۶/۳۰ ± ۱۵۶/۱۲ عدد در مترمربع) و دارای تفاوت معنادار بود ($P<0.05$). مقایسه آماری پراکنش مکانی این گونه در ۱۳ ایستگاه مورد بررسی نشان داد که ایستگاه‌های غربی با سایر ایستگاه‌ها تفاوت معناداری دارد ($P<0.05$). بر اساس آزمون همبستگی، بین میانگین تراکم این گونه با پارامتر محیطی ماده آلی کل و درصد سیلت در رسوبات بستر ارتباط همبستگی معناداری وجود نداشت.

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