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Effect of Storage on Various Honey Quality Parameters of Unifloral Sidder Honey from Pakistan*

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Abstract.- The sidder or ber honey of Pakistan can be stored for one year without seriously compromising with its quality. Although the values of various quality criteria had altered over a period of one-year; but they remained within the permissible limits. After one year of storage the pH varied between 7.4 and 6.8 for Bannu ber honey, 7.2 and 6.9 for Karak ber honey and 5.6 and 4.9 for Chunian ber honey. Similarly free acidity ranged between 6.5 and 29 meq/kg, 5.5 and 17meq/kg, 16 and 36.5meq/kg, lactone 0-2.7meq/kg, 1-7.5meq/kg, 0-2.5meq/kg, total acidity 6.5-31.7meq/kg, 6.5-24.5meq/kg, 16-39meq/kg, Electrical conductivity between 0.53-0.61mS/cm, 0.51-0.56mS/cm and 0.22-0.40mS/cm, HMF content between 2-11.2mg/kg, 1.7-10.5mg/kg, 4.6-26.9mg/kg, Proline content between 608.4-341.2mg/kg, 2800-476mg/kg, 621.2-147.3mg/kg, Diastase number between 33-23.4DN, 31-23.8DN, 42-27DN and Invertase number between 72.2-50.1, 68.7-52.9 and 90.27-44.7 for Bannu, Karak and Chunian ber honey, respectively.

Key words: Shelf life, ber honey, physicochemical characteristics of honey, proline content, HMF content, diastase number, invertase number.

Honey is susceptible to physical and chemical change during storage. It tends to be dark and loses its aroma and flavors (www.nhb.org). Honey is generally evaluated by a physico-chemical analysis of its constituents. Several of these constituents are of great importance to the honey industry as they influence the storage quality, granulation, texture, flavors and the nutritional and medicinal quality of honey. The International Honey Commission (IHC) has, therefore, proposed certain constituents as quality criteria for honey (Bogdanov *et al*., 1999). These include: moisture content, electrical conductivity, reducing sugars, amount of fructose and glucose, sucrose content, free acidity, total acidity, proline content, hydroxymethylfurfural (HMF), diastase activity, invertase activity and specific rotation. In the present study standard methods were used for determination of quality of honey. The purpose of the present study was to assess the effect of shelf life on physicochemical characteristics of sidder/ ber honey samples stored at ordinary room temperature for one year.

Materials and methods

Fresh Pakistani sidder honey samples were collected from Karak, Bannu and Chunian areas for study and stored under ordinary room temperature (25-29°C) in the laboratory until completely analyzed. There was no sign of granulation in honey samples. No preservative or any heating was applied at any stage.

All physicochemical determinations were essentially carried out according to the European Honey Commission methods (Bogdanov *et al*., 1999). pH and electrical conductivity were determined in a 10g/75ml solution of honey in deionized water. Free, lactonic and total acidities were titrated in the same solution used for pH measurement (AOAC, 1975). Water content was determined by refractive index and correlation with Chataway Charts (Chataway, 1935). Proline content of honey was determined according to Cough (1969). Winkler's method was used for the determination of HMF content (Winkler, 1955). The diastase and invertase activity of honey samples was determined according to the procedure of Schade *et al*. (1958) and Siegenthaler (1977), respectively.

Results and discussion

Table I show the effect of aging on shelf life of *Apis mellifera* honey from sidder collected from three different localities *viz*. Bannu, Karak and Chunian.

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Table I.- Effect of aging on physicochemical and biochemical parameters determining quality of shelf life of Sidder honey from Bannu, Karak and Chunian.

Month	pH	Free acidity (meq/kg)	Lactone (meq/kg)	Total acidity (meq/kg)	Electrical conductivity (mS/cm)	Diastase activity	Invertase activity	HMF	Proline content (mg/kg)
Bannu honey									
1st	7.4	6.5	0	6.5	0.53	33	72.2	2	608.4
2nd	7.4	8.5	0	8.5	0.53	31	68.4	2.3	593.8
3rd	7.2	9	0	9	0.53	31	66.9	2.8	578.9
4th	7.2	10	0.3	10.3	0.53	31	62.1	3.1	544
5th	6.9	10.1	0.5	10.6	0.54	31	62	3.7	506.1
6th	6.9	15	1	16	0.54	27	59.5	5.6	478.9
7th	6.9	15	1.5	16.5	0.56	27	57.7	5.5	471
8th	7.3	17	2	19	0.56	25	55	6.9	452.6
9th	7.2	18	2	20	0.57	25	52.9	7.2	398.7
10th	6.9	20	2	22	0.58	25	52	8.6	364.4
11th	6.8	25	1.5	26.5	0.59	24	50.1	9	360
12th	6.8	29	2.7	31.7	0.61	23.4	50.1	11.2	341.2
Karak honey									
1st	7.2	5.5	1	6.5	0.51	31	68.7	1.7	2800
2nd	7.2	6	1	7.5	0.51	31	68.7	2	2720
3rd	7.1	6.6	1	7.6	0.51	29	65.5	2.4	2112
4th	7.1	7.4	1.8	9.2	0.51	29	66.2	2.7	1598
5th	6.9	10	2	12	0.51	29	65	3	1098
6th	6.9	10.5	2	12.5	0.52	27.3	60.9	3.3	883.2
7th	6.7	11	2.3	13.3	0.52	27	59	3.9	769
8th	6.7	12.5	2.7	15.2	0.53	25	59	5.7	708
9th	6.7	14	4.1	18.1	0.53	25	58.8	6.8	700
10th	6.9	15	5.6	20.6	0.55	25.6	55.4	7.4	576
11th	7.1	16.5	6	22.5	0.55	24.1	55	9.2	528
12th	6.9	17	7.5	24.5	0.56	23.8	52.9	10.5	476
Chunian honey									
1st	5.6	16	0	16	0.22	42	90.27	4.6	621.2
2nd	5.3	19	0	19	0.22	40	88.5	9.0	744
3rd	5.1	20	0	20	0.22	40	83.15	11.5	877
4th	4.9	20.7	0.5	21.2	0.25	38	74.66	14.1	599.5
5th	4.9	23.5	1	24.5	0.31	32	73.6	16.3	546
6th	4.8	28	1	29	0.32	31	69	16.5	524
7th	4.3	30.2	1.2	31.4	0.32	31	60.3	16.9	472.6
8th	4.3	31	1.6	32.6	0.35	31	58.6	17.8	355
9th	3.3	32.5	1.8	34.3	0.35	30	57.1	20.6	269
10th	3.3	34	2.2	36.2	0.35	29	52.4	24.4	244.9
11th	4.7	34.8	2.5	37.3	0.40	27	45.8	26.2	158
12th	4.9	36.5	2.5	39	0.40	27	44.7	26.9	147.3

pH

The pH of the honey decreased during storage. The initial pH of Bannu, Karak and Chunian ber honey was 7.4, 7.2 and 5.6 respectively (Table I), which after six month of storage decreased 6.7%, 4.16% and 14.2%, respectively. Although the initial pH of three types of ber honey was different from each other, they showed similar behavior of decrease during storage. In the following six months this decline was 2.08% in Chunian, 1.44% in Bannu sidder honey, while Karak ber honey's pH remained unchanged. Overall, after 12 month's of storage the pH of three types of honeys decreased 8% for Bannu, 4.16% for

Karak and 12.5% for Chunian honey. These values were however, within the international limits acceptable for honey for table use.

Free acidity, lactone and total acidity

A gradual accumulation of acids in Pakistani sidder honey indicated a uniform effect of aging. Free acidity increased 346% (4.45 folds) for Bannu, 209% (3.09 folds) for Karak and 128% (2.28 folds) for Chunian honey samples after one year of storage. During the first six months, this increase was 130% (2.3 folds) in Bannu, 90.9% (1.9 folds) in Karak and 75% (1.75 folds) in Chunian samples. During the next 6 months period this increase was 93% in Bannu, 61.9% in Karak and 30.55% in Chunian samples. Anyhow, the free acidity was still within the permissible limit of 40mg/kg.

Similarly lactone formation also increased tremendously in the stored samples. After one year of aging the lactone content had increase 800% (9 fold) in Bannu, 650% (7.5 fold) in karak and 400% (5 fold) in Chunian. Total acidity is the sum of free acidity and lactone. The increase in total acidity during initial six months was 146% in Bannu, 92% in Karak and 81.2% in Chunian honey samples. After a year's storage this total acidity increased, respectively 387% (4.57 fold), 277% (3.77 fold) and 143% (2.4 fold). A similar trend of increase in total acidity has also been observed by Cervantes *et al*. (2000) who first heated the honey samples at 55ºC and then kept those samples at 26ºC for three and half months.

Electrical conductivity

During storage, the electrical conductivity (EC) increased in sidder honeys. It showed maximum increase of 82% in Chunian, 15% in Bannu and 10% in Karak honey samples at the end of one year's storage. In any case the conductivity remained within the prescribed limits for fresh honeys.

Diastase activity

The diastase activity decreased during storage, but inspite of that it remained within the permissible limits. The diastase number of Bannu, Karak and Chunian honey samples was respectively 33, 31 and 42 before storage. During the first six months the diastase activity decreased 18%, 12% and 20% in Bannu, Karak and Chunian honey samples. It decreased 29%, 23% and 36%, respectively after storage for one year. Similar trend was noticed by White and Subers (1963) and Iman (1990). Sancho *et al.* (1992) reported depletion in diastase activity after studied the effect of storage for two years at 20°C. Cervantes *et al.* (2000) also reported decrease in diastase activity after heating the honey at 55°C and then storing it for three and half months at 26°C. Castro-Vázquez *et al*. (2008) also reported that diastase activity was out of the legal limit in citrus honey stored for 12 months at 40ºC.

Invertase activity

Being more sensitive to aging, the deterioration of invertase per month was faster as compared with that of diastase. It started right from the first month of storage. The original value of invertase depends on the origin of honey which shows a great variation. Initially Bannu and Karak sidder honey samples showed invertase number of 72.2 and 68.7, respectively, as compared with Chunian honey sample which had 90.27. But Bannu and Karak sidder honey also showed less decline in invertase activity, 31% and 23% respectively, as compared to Chunian honey sample which showed 51% decrease at the end of one year storage. The decrease in invertase activity has also been studied by Takenaka and Echigo (1974) and Ivanov (1981) after six months, White (1964) after eighteen months, by Krauze and Krauze (1991) and Sanchez *et al.* (2001) after twenty-four months.

HMF Content

During storage the change in the HMF content in sidder honey samples was due to the continuous accumulation of HMF in stored samples. The maximum increase of 518% in HMF content was shown by Karak ber honey followed by 485% in Chunian and 460% in Bannu ber honey after twelve months storage. The average monthly increase in HMF was found higher in Karak sample than in Bannu and Chunian honey samples. Increase in HMF was 180% in Bannu, 94% in Karak and 259% in Chunian honey samples during first six months of storage and 100% in Bannu, 218% in

Karak and 63% in Chunian ber honey during the last six months of storage. The same significant increase in HMF content of honey has also been observed in other laboratories (Iman, 1990; Sancho *et al*., 1992; Cervantes *et al.*, 2000; Sanz *et al.*, 2003; Castro-Vázquez *et al*., 2008).

Proline content

The proline content were depleted during storage. During initial six months the reduction in proline content was 21% in Bannu, 69% in Karak and 16% in Chunian sidder honey samples. After a year's storage decline in proline was 83% in Karak followed by 76% in Chunian and 43% in Bannu samples.

Conclusion

The physiochemical characteristics of the unifloral sidder honey of Pakistan were well within the limits prescribed for good quality exportable honey by the International honey quality standards. The sidder / ber honey of Pakistan can be stored for about one year with seriously compromising its quality.

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Quality Assessment of Fresh Milk Obtained From Different Sources

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Abstract.- The fresh milk of buffaloes, cows and goats were analyzed for fat, protein, lactose, total solids, solids not fat, total nitrogen, non protein nitrogen, total titratable acidity, pH, lactometer reading and trace metals contents. Non significance difference of nutrients within the samples but comparable with standard values were observed. The level of fat in milk of buffalo, goat and cow was 6.2, 5.8 and 5.2%, respectively, where as protein in milk of goat, cow and buffalo was 4.8, 4.2 and 3.6%, respectively. The lactose in milk of goat, cow and buffalo constituted 4.8, 4.3 and 3.9%, respectively. Total nitrogen level in buffalo, cow and goat milk was 0.64, 0.61 and 0.55%. The level of solids not fat in these milk samples followed the order of cow > goat > buffalo. The level of Na followed the gradation, buffalo (40.5 ppm) > goat (39.2 ppm) > cow (38.1 ppm), whereas concentration of Ca also followed the order of buffalo (19.8 ppm)> goat

(19.6 ppm)> cow (19.1 ppm). Quality of milk depends on its sources however, storage of milk for long durations requires its proper treatment.

Keywords: Raw and processed milk, nutritional factor, milk quality assessment.

Milks of mammals and particularly of livestock including cows, buffaloes, sheep, goats and camels provide primary source of nutrition for newborn mammals before they are able to digest other types of food. The components of raw milk are influenced by breed of animals. It was reported elsewhere that breed producing milk with high fat content produce less milk than those with lower fat. Composition of milk also varies with stage of lactation and early stage of lactation is colostral state, rich in antibodies and different from normal milk in color and taste (Rashida *et al*., 2004). Milk contains significant amounts of saturated fat, protein and calcium as well as vitamin C. Cow's milk has a pH ranging from 6.4 to 6.8, making it slightly acidic. In many cultures of the world, especially the Western world, humans continue to consume milk beyond infancy, using the milk of animals as a food product (Harwalkar, 1982). Raw, processed liquid milk in the form of pasteurized or ultra heat treated milk is the main dairy product in Pakistan while other products include dry powdered milk, cream, butter, butter oil, yogurt, cheese and ice cream (Ali *et al*., 1980; Andrew and Cheeseman, 1971).

Casein protein micelles are largest structures in the fluid portion of the milk (Holt, 1995; Henle *et al.,* 1996), which collectively make up around 80 percent of the protein in milk, by weight.

Pakistan ranks 3rd amongst milk producing countries with 38.37 million tons per year (GOP, 2007), out of which 71% is produced by buffaloes, 24% by cattle and 5% by sheep and goats (Rashida *et al*., 2004). In Pakistan milk is processed by milk industry to increase its shelf life without compromising its nutritive value. However, poor milk quality because of milk adulteration and high somatic cell count is a big problem of milk industry. People are consuming both fresh and processed milk to fulfill their requirements and it is important to maintain quality of milk for the health of people. The present study was therefore conducted to determine the quality of important nutrients of fresh milk of buffaloes, cows and goats to assess the quality of the milk.

Materials and methods

A total of 45 fresh milk samples of buffaloes, cows and goat, 15 each, were collected in sterilized bottle directly from forms of milk supplier from rural areas of Rawalpindi Region. Milk samples were immediately transported to BCH laboratory (PMAS Arid Agriculture University Rawalpindi) for analysis of different parameters. Lactometer reading and specific gravity of milk samples were determined according to Eckles *et al*. (1957) and Lampert (1965). The pH was determined by a pH meter (Consort C 833, Multi parameter analyzer). Acidity test of milk samples was done according to AOAC (2000).

The total fat content was determined according to AOAC (1990). Lactose sugar was estimated according to Patel and Mistry (1997), amount of solids not fat was calculated according to David (1977) and % content calculated according to Kanwal *et al*. (2002), the total solids were determined according to AOAC (1990), and total protein was determined by Kjeldhal method (AOAC, 2000).

For determination of mineral content 2 g of milk sample was taken in a flask to which 10 ml of nitric acid was added and heated at 60-70^{o}C for 15 min, until solution became clear. Then 5 ml of perchloric acid was added and heated at 80^{o}C for 15 minutes and boiled vigorously, until volume was reduced to 1-2 ml. Reaction mixture was cooled and volume was raised up to 10 ml by diluting it with double distilled water, which was then used for estimation of Na^{+}, K^{+}, Ca^{++}, Mg^{++}, Fe^{+++}, Cu^{++} and Zn^{++} ions using flame atomic absorption spectroscopy (Richards, 1968).

Data was analyzed statistically by using analysis of variance (one way ANOVA) and were presented as Mean±S.D. Differences of the mean were considered to be significant when $p< 0.05$.

Results and discussion

Table I shows the physical and biochemical parameters, whereas Table II shows metal ion content of fresh milk of buffaloes, cows and goats.

Table I.- Compositional analysis of different types of milk.

	Buffalo milk (n=15)	Cow milk (n=15)	Goat milk (n=15)	Standard
Physical parameters				
Lactometer reading	26.6± 0.5	27.1± 1.2	28.2± 1.4	-
Specific gravity	1.01± 0.0	1.02± 0.0	1.03± 0.0	1.03± 0.1
Total titrable acidity (%)	0.11± 0.0	0.13± 0.0	0.16± 0.0	0.15± 0.1
pH	6.4± 0.05	6.5± 0.3	6.3± 0.1	6.4± 0.5
Chemical composition				
Fat (%)	6.2± 0.5	5.2± 0.2	5.8± 0.4	4. 50± 0.47*
Protein (%)	3.6± 0.3	4.2± 0.4	4.8± 0.6	3.13± 0.16
Lactose (%)	3.9± 0.5	4.3± 0.7	4.8± 0.5	4.59± 0.16
Total nitrogen (%)	0.64± 0.0	0.61± 0.0	0.55± 0.1	-
Solid non fat (%)	8.4± 0.8	8.6± 0.2	8.5± 0.1	8.42± 0.20
Non protein nitrogen (%)	0.003± 0.0	0.004± 0.0	0.004± 0.0	-
Total solids (%)	12.1± 0.5	13.2± 0.8	12.5± 0.6	12.92± 0.54

Mean ±SD on the basis of triplicate analysis, p <0.05. *Standard source Yooyuenyong *et al*. (2003).

The specific gravity, total titrable acidity and pH of the tested milk sample were similar to the standard values. The titratable acidity test also did not show any significance difference in the milks of buffaloes, cows and goats. Campbell and Marshall (1975) found maximum value of acidity in milk (0.23%). In the present study value of acidity found in goat milk was 0.16 followed by cow 0.13 and buffalo milk 0.11. When the pH of milk is changed, the acidic or basic groups of the proteins will be neutralized. If an acid is added to milk, or if acid producing bacteria are allowed to grow in milk, the pH falls, causing casein precipitation.

The concentration of fat in buffalo milk was 6.2 % followed by goat milk (5.8%) and cow milk (5.2 %) (Table I). These findings are similar to those reported by Rashida *et al*. (2004) from similar types of milk of from Rawalpindi and Islamabad areas. Some studies revealed that about 98% of milk fat is a mixture of triacyl glycerides, which further include neutral lipids, fat soluble vitamins and pigments (*e.g.* carotene), sterols and waxes. Milk fat acts as a solvent for the fat soluble vitamins A, D, E and K and also supplies essential fatty acids like linoleic, linoenic and arachidonic acids. Total solid contents found in the milk samples of cow, goat and buffalo were 13.5, 12.5 and 12.1%, respectively. Similar values in milk were also reported by Gervilla *et al*. (1997). The total solids of milk other than fat (SNF), showed value of 8.6% for cow milk followed by goat (8.5%) and buffalo milk (8.4%) (Table I).

Table II.- Analysis of metal ions (ppm) of different types of milk.

	Buffalo milk (n=15)	Cow milk (n=15)	Goat milk (n=15)
Na^{++}	40.5±2.5	38.1±1.5	39.2±1.3
K^{++}	11.7±0.5	12.4±0.4	12.3±0.1
Ca^{++}	19.8±2.1	19.1±3.3	19.6±1.5
Mg^{++}	6.4±1.1	6.5±0.8	6.3±0.5
Fe^{+++}	3.4±0.8	3.1±0.6	3.9±0.4
Cu^{++}	1.2±0.5	1.1±0.2	0.8±0.0
Zn^{++}	6.4±1.1	6.2±1.2	5.8±1.3

Mean values ± SD on the basis of triplicate analysis p <0.05

The milk samples of goat, cow and buffalo showed 4.8% protein in goat followed by cow milk (4.2%) and buffalo (3.6), whereas TN was 0.64% in buffalo milk followed by cow milk (0.61%) and goat milk (0.55%). Non significance differences in milk proteins of buffalo, cow and goat have also been reported by Rashida *et al*. (2004) and Louis (1970). Normally nitrogen content of milk is distributed among caseins (76%), whey protein (18%) and non protein nitrogen (NPN (6%) as reported by Puerto *et al*. (2004).

The lactose contents of goat milk was found to be 4.8% followed by cow (4.3%) and buffalo (3.9%). Under controlled conditions lactose can be fermented to propionic acid to give a desired flavour. The variations in lactose content of various milk samples were also reported by others (Rashida *et al*., 2004; Hanjra *et al*., 1989).

Table II shows concentration of metal ions like Na^{+}, K^{+}, Ca^{++}, Mg^{++}, Fe^{+++}, Cu^{++} and Zn^{++} in fresh milk samples of buffaloes, cows and goats. No significant differences was observed when

concentration of these metals were compared with each other. Franco *et al*. (2003) reported non significant differences in Ca, Fe and Cu and other metals from milk and cheese samples. They further revealed that losses of these metals in the whey balance the tendency to increase their concentration for the normal drying during ripening process. However, some milk products had higher Na, K and Mg concentration than some other fresh cheese. Furthermore higher amounts of Zn lost probably due to consequences of its association with albumins and other proteins of milk. All nutrient parameters found in these milk samples are comparable to standard values reported by Yoouenyong *et al*. (2003).

Finally it is concluded that fresh milk of all the three breeds (buffaloes, cows and goats) contain all essential nutrients like protein, lactose, fat and essential metal ions, comparable to the standard values reported in literature. However, alteration in milk composition are possible due to adulteration, improper handling and poor storage conditions of milk.

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Food Habits of the Indian Crested Porcupine, *Hystrix indica* in Sindh, Pakistan

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Abstract.- The fecal pellets of *Hystrix indica* were collected from the agricultural lands of Malir in the suburbs of Karachi. *Hystrix indica* mainly fed on agricultural crops including vegetables and fruit rather than xeric vegetation. Diet of Porcupine was observed to

vary and comprised of 66.7% vegetable matter. Tuber/roots, leaves /stem and fruit formed 3.9%, 46.3% and 16.5% of the diet consumed respectively. Agricultural crops like *Cucurbita maxima* (Pumpkin), *Psidium guajava(Guava) and Solanum melongena* (Brinjal) were eaten extensively. It has been observed that *Hystrix indica* is the serious pest of agricultural crops of the area. Long term studies are proposed for better understanding of food habit of this important pest of crops, forestry and rangeland.

Key words: *Hystrix indica,* fecal pellets, cropland, Sindh.

The Indian crested porcupine, *Hystrix indica* is an old world Porcupine species and is a rodent pest of economic importance in agriculture and forestry system in Pakistan (Khan *et al.,* 2000). Besides it is also a generalist forager that exploits a wide variety of cultivated and wild plants and consumes above ground as well as sub-surface plant tissues (Gutterman, 1982). The crops of economic importance such as maize, groundnut and potato are heavily attacked by porcupine in field areas of the Punjab and Balochistan provinces. Ahmed *et al*. (2003) recorded upto 30% damage to neem plantation in rangeland of lower Sindh. Similar damage was observed to Saffron (*Crocus sativa*) plantation at Mastung, Balochistan. Khan and Mian (2008) reported 30-70% damage to Gladiolus and Dutch Irish plantation in a floriculture farm, Islamabad. In spite of its status as a serious agriculture and forestry pest, no study of its food and feeding habit in cropland in Sindh has been conducted. Here in, we report data of six month study of porcupine diet based on fecal pellet analysis in farm land of Malir, Karachi.

Materials and methods

The study area, the Haji Adam Jokhio farm, is located in the district Malir, Karachi (24°,45′ N, 67°,09′E) opposite to the coastline of Arabian sea and 10 km off National Highway. The land soil consists of loamy clay and somewhat sandy soil irrigated by tube well. Basically, it is an arid area comprised of six kilometer in radius, surrounded by hills and covered with sparse vegetation. Being located in the suburb of city, vegetables are the main produce of the farm.

In study area, 41 different plant species including diverse plants of xeric vegetation were recorded and 17 different plant species were identified from fecal pellet analysis. During a six months period, bimonthly visits were made for fecal pellet collection in the fields and adjacent hilly areas. Due to denning of porcupine in inaccessible area and uneven terrain, only 11 samples of 10 gm each could be collected.

In the laboratory, fecal pellets were washed over three consecutive sieves (mesh size 2mm; 1 mm and 0.5 mm). About 15 sub-samples were randomly taken from 2 mm and 1 mm sieve each and 100 fragments identified at 10x magnification. The contents of 0.5 mm sieve were examined at 100x magnification by making four slides according to the procedure of Hansen *et al.* (1971). Identification was made by comparing the characteristic of fragment with reference to plant material. On each slide, 5 locations of 2mm in diameter each were chosen randomly and fragments were identified. A sample of at least 300 fragments of the contents of each 10 gram fecal pellets was thus examined.

For reference study, 17 plants species were prepared by following the above mentioned procedure. Identification of plant fragments were made on the basis of cellular characteristic including structure and size, variation of stomata, epidermal cellular characteristics, presence or absence of silica bodies and silica suberose bodies.

The frequency of each food item in each sieve was expressed as a percentage (x) of the total fragments in the sample of that sieve. The contents of each sieve were dried and weighted and the percentage (y) of the total dry weight of the fecal pellets retained by each sieve was calculated. The quantity of each food item could then be expressed as a percentage of the total pellets contents:

$$\text{Percentage for a food item: } \Sigma: \frac{Xi\ Yi}{100}$$ where (i = 1, 2, 3 for each sieve)

Results and discussion

Data of Table I showed that there was great diversification in respect of plant flora comprising

Table I.- Number of samples in which various plant species were identified in diet of *H. indica*.

Plant species identified	Common/Local Name	Leaves/Stem	Fruits/Seeds	Tubers/roots
Acacia senegal	Gum arabic, Khor, Kher	4	0	0
Aeluropus lagopoides	-	1	0	0
Azadirachta indica	Neem	7	0	0
Capparis deciduas	Kurrel, Karer	4	0	0
Commiphora wightii	Guggal, Salaitree, Gum-guggal	11	0	0
Cucurbita maxima	Pumpkin	9	3	0
Cynodon dactylon	Barmuda grass, Doob grass	2	0	6
Euphorbia caducifolia	Danda thore	11	0	0
Lycopersicum esculentum	Tomato	2	10	0
Murraya koenigii	Curry leaf	1	0	6
Prosopis cineraria	Jhand, Jand, Kandi, Cikura	4	0	8
Psidium guajava	Guava, Amrud	8	9	0
Solanum melongena	Brinjal	0	7	2
Terminalia catappa	Indian almond, Jangli badam	6	0	0
Trcohodesma amplexicaule	-	2	0	0
Withania somnifera	Asgandh, Winter cherry	1	0	0
Zygophyllum simplex	Alethi, Putlani	3	0	0

Table II.- Analysis of fecal pellets of *Hystrix indica* showing percent of total contents for different food items in each sample.

Sample No.	Leaves	Stems	Seeds/fruits	Tubers/roots	Insects	Stone pieces	Unidentified animal matter	Unidentified vegetable matter	Total identified matter
1	24.5	19.2	22.3	4.0	6.8	6.5	3.1	13.6	83.3
2	24.5	21.8	14.4	6.0	6.0	7.4	2.4	17.5	80.1
3	26.6	17.5	16.9	6.2	5.2	10.1	1.0	16.5	82.5
4	24.5	21.0	14.7	5.4	9.4	7.3	1.6	16.1	82.3
5	26.3	23.0	15.0	5.7	7.0	6.3	2.7	14.0	83.3
6	25.3	18.7	17.7	0	6.0	8.3	5.0	19.0	76.0
7	26.0	24.0	15.7	2.3	7.3	9.3	2.3	13.0	84.6
8	27.0	24.3	11.3	6.3	7.8	12.0	0	11.3	88.7
9	26.0	21.7	17.3	0	10.0	7.0	0	18.0	82.0
10	26.4	18.7	16.3	5.0	9.0	8.0	0.3	16.3	83.4
11	28.0	14.7	20.3	2.0	10.0	2.6	5.7	16.7	77.6
Mean	25.9	20.4	16.5	3.9	7.6	7.7	2.2	15.6	82.2
SD	1.13	3.0	2.9	2.4	1.7	2.4	1.9	2.3	3.4

of agriculture and Xerophetic fauna. A total of 17 plant species could be identified. *Cucurbita maxima*, *Azadirachta indica; Solanum melongena, Lycopersicum esculentum; Psidium guajava and Euphorbia caducifolia* were eaten most often and accounted for all fruit seeds and leaves in the fecal pellets. The roots/tubers of the *Prosopis cinerania* and stem of *Commiphera wightii* and *Euphorbia caducifolia* were also eaten frequently. All the eleven samples has stem of *Euphorbia caducifolia* and preference were given over to fruits of *Lycopersicum esculentum*. Table II shows the different food items as percentages of the total for each sample. Vegetable matter formed 66.7% of the fecal contents mostly leaves / stem (46.3%) fruit and seed (16.5%) and tuber or roots (3.9%). Unidentified animal matter was also found in nine samples and formed an average 2.2% of the fecal contents, ranging from 0.3-5.7%. Identification of animal matter was sometimes impossible. Insects

were found in all eleven samples tested in laboratory (mean: 7.6, range: 5.2-10.0). Plant uprooting sites were numerous around and near the porcupine dens from where pellets were collected. Typically many uprooting sites occurred closed together, so the soil seemed to be ploughed to excavate the roots / tuber of plants. Often fragments of *Euphorbia cauducifolia* have been observed scattered at spots. The soil insects alongwith stone pebbles were also identified during pellet analysis study which might have been consumed by *Hystrix indica* during nocturnal foraging.

The results of the present study showed that *H. indica* in crop land and range land areas of Malir is largely herbivorous in diet and serious pest of seasonal vegetables, fruits, flowering plants and grasses. The study also confirmed the findings of Pervez *et al*. (2005) where stem of *E*. *caducifolia* was noted to be the preferred food item. *E*. *caducifolia* is a bushy thorny plant belonging to Cactus family having juicy stem and is highly susceptible to porcupine damage. Chewed parts of plants have also been collected near and inside the animal den. All the sample of pellets analyzed had stem pieces of *E. caducifolia.* Furthermore, the study revealed that *H. indica* is also a serious pest of tomato, brinjal, gourd and guava in Malir orchards, Sindh.

A detail study on feeding preference of *H.indica* through application of three possible approaches *i.e.* fecal sample analysis, stomach analysis and damage plants analysis is suggested to overcome the apparent difficulty of collection of fecal samples.

Conclusion and recommendations

From this feeding study of *H. indica*, it is obvious that it is largely herbivorous in diet and serious pest of seasonal vegetable, fruit, flowering plants and grasses. Our results are based on small sample of food pellets collected over a six month period. To ascertain seasonal variation in diet, however, a long term study would be necessary.

The seasonal changes in temperature and rainfall undoubtedly influence the abundance and diet of *H. indica* resulting in intensification of crop losses. For proper management, burrow fumigation through aluminum phosphide (3 g) tablets followed by second generation anticoagulant bait is recommended. A sustained pest vigilance coupled with constant monitoring is suggested to check for any recurrence of the pests after implementing control measures.

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Occurrence of Metazoan Parasites of the Mirror Carp (*Cyprinus carpio* L. 1758) in a Fish Farm, Uluabat, Bursa, Turkey

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Abstract.- Metazoan parasite of the mirror carp (*Cyprinus carpio* L.) in a fish farm ponds, Bursa, Turkey was investigated from April 2005 to March 2006. Sixty four out of 79 mirror carps were infected with one or more of the four parasite species - *Dactylogyrus extensus*, *D. anchoratus* and *Paradiplozoon homoion* (Monogenea) on the host gills and *Argulus foliaceus* (Crustacea) on oral cavitiy, gills, fins and skin. *D. extensus* was the most frequent having an overall prevalence of 39.2%. *P. homoion* was 54.4%. The third most prevalent parasite was *D. anchoratus* with

overall prevalence of 25.3%. *A. foliaceus* showed overall prevalence of 43.03%. At the end of this study, when *P. homoion* was recorded as parasite species being the highest overall prevalence, *D. extensus* was observed in the highest intensity. *D. extensus*, *D. anchoratus* and *P. homoion* were recorded for the first time in the mirror carp in Turkey.

Key Words: Mirror carp, *Cyprinus carpio*, Metazoan parasites, *Dactylogyrus extensus*, *D. anchoratus*, *Paradiplozoon homoion, Argulus foliaceus*

Common carp originally from Asia was introduced to Turkey at the beginning of 1900 and has become established at large. Although the helminth parasites of the common carp in Turkey have been extensively, only limited studies are available for parasites of the mirror carp (Anonymous, 1985; Timur *et al*., 1993; Cengizler *et al*., 2001; Tabakoglu *et al*., 2004). Among the ectoparasites of the mirror carps, two species of monogenean parasites, *Dactylogyrus vastator* and *Gyrodactylus elegans* were recorded at the Adana region (Tabakoglu *et al*., 2004) and Seyhan river (Cengizler *et al*., 2001). Two species of Crustacea, *Lernea cyprinacea* and *Argulus foliaceus* were collected at the Adana region (Tabakoglu *et al*., 2004), Seyhan river (Cengizler *et al*., 2001) and Anonymous (1985). As far the endoparasite fauna, species of three genera of Cestoda, *Bothriocephalus* sp. at Akşehir region (Timur *et al*., 1993), *Schistocephalus* sp. and *Caryophylaeus* sp. from Seyhan river (Cengizler *et al*., 2001).

The present study focuses on the occurrence of the metazoan parasites fauna of mirror carp in a fish farm pond, Turkey.

Materials and methods

Mirror carp was obtained from a fish farm is located around 200 m to the north of Lake Uluabat located at 41° 11' N, 29° 4' in northwest Anatolia. The fish farm consists of three big rectangular ponds (95 m x 65 m x 1 – 3 m), water supply of which was received from the lake Uluabat via –a human – made canal. A total of 79 mirror carps were monthly captured during the period from April 2005 to March 2006 using gill net, cast nets or creel. But, no fish was sampled during August, December, January and March and transported alive in an aquarium in the laboratory. Within 24 hours, fish were killed by vertebral dislocation, and dissected to examine the viscera, gills, gastrointestinal tract, liver, kidney, heart, swim bladder, gallbladder, eyes, fins and body surfaces under a dissecting microscope. All parasites found in each individual fish were identified and counted. Species were identified according to the keys of Markewich (1951), Bykhowskaya – Pavloskaya *et al*. (1962), Gussev (1985) and Gussev *et al. (*1987), whereas prevalence (%) and mean intensity of parasites were determined according to Bush *et al*. (1997).

Results

Of the 79 mirror carp examined, 64 were infected with four species of parasites, three Monogenea and one crustacean. Table I shows prevalence and intensity of parasites found in mirror carp. *Paradiplozoon homoion* was recorded as parasite with highest overall prevalence and *Dactylogyrus extensus* as of highest intensity. Of 79 fish examined, 31 were infected with *D. extensus* with overall prevalence of 39.2%. A total of 1309 parasites were found on 31 fish. The prevalence of *D. extensus* increased from April to July. It peaked in May and June (Table I). The highest intensity was observed in May, whereas the lowest intensity was recorded in April. This species was not detected in the months September – November and February.

P. homoion has the highest prevalence (54.4%). It had 70 – 100% prevalence throughout the year with the exception of May and June when they decreased to 8.3% and 40%, respectively (Table I). This species was not detected in the month of April. Changes in intensity of *P. homoion* followed a seasonal pattern, the highest intensity was observed in June, whereas the lowest intensity was recorded in the month of May (Table I).

A total of 206 specimens of *Dactylogyrus anchoratus* were found on 20 of 79 fish examined, with overall prevalence of 25.3%. As can be seen in Table I, this species was recorded from April to June, with 90% prevalence in June. This species was not detected in the months of July, September to

Table I.- Prevalence, intensity and infection details of metazoan parasites recovered from monthly samples of mirror carp in fish farm ponds.

	April (n=12)	May (n=12)	June (n=10)	July (n=6)	Sept. (n=10)	Oct. (n=10)	Nov. (n=9)	Febr. (n=10)	Total (n=79)
Infection with *D. extensus*									
Prevalence (%)	33.3	100	100	83.3	0	0	0	0	39.2
Intensity range (Min - Max)	1-7	4-107	3-86	43-95	0	0	0	0	1-107
Total infection	19	665	306	319	0	0	0	0	1309
Infection with *D. anchoratus*									
Prevalence (%)	8.3	83.3	90	0	0	0	0	0	25.3
Intensity range (Min - Max)	1	3-18	3-22	0	0	0	0	0	1-22
Total infection	1	100	105	0	0	0	0	0	206
Infection with *P. homoion*									
Prevalence (%)	0	8.3	40	100	70	80	77.7	100	54.4
Intensity range (Min - Max)	0	2	1-3	5-51	1-13	1-6	1-9	3-16	1-51
Total infection	0	2	8	132	33	29	34	68	306
Infection with *A. foliaceus*									
Prevalence (%)	50	91.6	100	83.3	20	0	0	0	43.03
Intensity range (Min - Max)	1-4	1-10	1-18	1	1-21	0	0	0	1-21
Total infection	11	49	77	5	23	0	0	0	165

No sampling was done in the months of August, December, January and March.

November and February. The infection of *D. anchoratus* occurred in April (only one specimen) and rapidly increased in May and June. The highest intensity was observed in June (Table I).

A single crustacean species, *A. foliaceus* was found on oral cavity, gills, fins and skin of the host fish. *A. foliaceus* was observed in 34 of the 79 fish examined. A total of 165 parasites were recorded, with an overall prevalence of 43.03% (Table I). The infection levels for this parasite were erratic with prevalence varying between 100% in June and 20% in September. No infected fish were found in October, November and February. The infection of this species occurred in April and rapidly increased in May and then the infection peaked in June (Table I).

Discussion

The most common parasite of *C. carpio* is *D. extensus*, which is a host specific. This parasite has been reported previously in *C. carpio* in Turkey by many authors (Oguz *et al*., 1996; Aydogdu *et al*., 2001; Aydogdu and Altunel, 2002a; Özturk, 2005; Öztürk and Altunel, 2006; Özturk and Bulut, 2006; Uzunay and Soylu, 2006; Soylu and Emre, 2007). Aydogdu and Altunel (2002b) recorded this parasite as a dominant species in *C. carpio* with a prevalence of 69.7% in Lake Iznik. Similarly, Aydogdu et al. (2001) recorded 31 common carp out of 43 infected with a total of 704 parasites. Soylu and Emre (2007) reported 23.6% prevalence of this parasite in *C. carpio* from Kepez, Antalya. In a similar study, Öztürk and Altunel (2006) recorded 41.5% prevalence of this species in *C. carpio* and an infection intensity of 20.2 in Lake Manyas. In addition, Oguz *et al*. (1996) in Lake Uluabat, Aydogdu and Altunel (2002a) in Dogancı Dam Lake, Özturk (2005) in Lake Eber, Özturk and Bulut (2006) in Dam Lake Selvir and Uzunay and Soylu (2006) in Lake Sapanca recorded *D. extensus* from *C. carpio* with a prevalence 90%, 100%, 73.6%, 72.5% and 75%, respectively. Our findings are similar to those of Öztürk and Altunel (2006).

D. anchoratus was found to be the third most common parasite of *C. carpio* with 25.3%

prevalence. *D. anchoratus* is a host specific parasite of both common carp (*C. carpio*) and Crucian carp (*Carassius carassius*). To our knwledge, *D. anchoratus* was recorded in Crucian carp from Lake Kovada by Ozan and Kır (2005), from Golbasi Dam Lake by Aydogdu (2006), from Enne Dam Lake by Koyun and Altunel (2006), from Çobanlar (farm) and Lake Bektaşaga in Common carp by Özer (2002), from Enne Dam Lake in Golden carp by Koyun and Altunel (2006). As to the infection results of this species in Turkey, Ozan and Kır (2005) recorded that 29 *C. carpio* out of 102 were infected with this parasite, and 275 *D. anchoratus* were recorded in them. The infection was highest in March (52.9%) and lowest in December (11.1%). In a different study, Koyun and Altunel (2006) recorded this parasite both in Crucian carp (*C. carassius*) and G. carp (*C. auratus)* with a prevalence of 22.67 and 22.68% in Enne Dam Lake, respectively. Özer (2002) studied co–existence of *D. anchoratus* and *D. extensus* in *C. carpio* from the two localities in nothern Turkey, and reported a prevalenve of 21.8% at Çobanlar (farm) and 34% at Lake Bektaşaga for this parasite. The infection reached its peak in the winter. In a similar study in Turkey, Aydogdu (2006) recorded in 48 out of 132 *C. carpio* in Golbası Dam Lake, with overall prevalence of 36%. In his study, the prevalence levels of this species were somewhat erratic and its infection varied according to the season ad reached its highest values in May (100%). Our data on the prevalence of *D. anchoratus* in *C. carpio* from fish farm ponds is very similar to that reported by Özer (2002) and Koyun and Altunel (2007) for this species. The overall prevalence of this species was 25.3% in our study, 21.8% in Özer's (2002) study and 22.67% in Koyun and Altunel's study (2007).

In the present study, *P. homoion* was found to be the second most dominant parasites of the mirror carp with 54.4% prevalence. To our knowledge, there are 3 reports of *P. homoion* from Turkey. This species was recorded in *Alburnus alburnus* from Enne Dam Lake by Koyun and Altunel (2007), from Lake Manyas in *Rutilus rutilus* by Öztürk (2000) and from Kepez, Antalya in *Pseudophoxinus antalyae* and *C. carpio* by Soylu and Emre (2007). Koyun and Altunel (2007) studied metazoan parasites of Bleak (*A. alburnus*) in Enne Dam Lake druring two years and they sampled 341 fish specimens and recorded 440 *P. homoion* in 102 of 341 Bleak. This parasite was found throughout the two years with the exception of February and March 98. They reported a prevalence of 30% for this parasite in their study. Soylu and Emre (2007) examined the monogenean parasites of *C. carpio* and *P. antalyae* living in the Antalya region, Turkey. They reported that 53 *P. antalyae* out 72 were infected with this parasite (overall prevalence 73.6%) and also found one specimen of *P. homoion* on the gills of *C. carpio*. The record of *P. homoion* in the mirror carp provides a further example.

A single species of Crustacea, *A. foliaceus* was observed in 34 of 79 host fish with overall prevalence of 43.03%. As indicated by Öktener (2003), *A. foliaceus* was recorded in *Cyprinus carpio*, *Leuciscus cephalus*, *Tinca tinca*, *Alburnus alburnus*, *Silurus glanis*, *Capoeta trutta, Esox lucius*, *Carassius carassius* in Turkey. The record of *A. foliaceus* in the mirrror carp contributes to increase our knowledge on the occurrence of this parasite in a different locality.

In conclusion, the results of the present study contribute to increase our knowledge on the occurrence of parasites of the mirror carp in a different locality. At the same time, as far as we know *D. extensus*, *D. anchoratus* and *Paradiplozoon homonion* were recorded for the first time in mirror carp in Turkey.

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A Sipunculan Worm, *Themiste (Lagenopsis) lageniformis* (Baird, 1868) (Golfingiiformes: Themistidae) New to Pakistani Waters

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Abstract.- During routine collection trips a few Sipunculan worms *Themiste (Lagenopsis) lageniformis* (Baird, 1868) were collected from the coastal waters of Pakistan. This is the first report of the species collected along the northern Arabian Sea (Pakistan). It is here briefly described and illustrated with a note on its distribution ecology and habitat.

Key Words: Sipuncula, new record, Pakistan.

Sipunculans are exclusively marine unsegmented worms. Based upon the classification of Cutler (1994), there are two classes of Sipuncula *i.e.,* Phascolosomatidea and Sipunculidea. The former comprises two orders, the Phascolosomatiformes and Aspidosiphoniformes, each with a single family, the Phascolosomatidae and Aspidosiphonidae, respectively. The class Sipunculidea is composed of two orders-the Sipunculiformes and Golfingiiformes. The former is composed of the single family Sipunculidae while the latter includes the three families, Golfingiidae, Phascolionidae and Themistidae.

The Sipunculan fauna of the Indian coast represents as many as 37 species and subspecies in 10 genera under 5 families (Halder, 1991). A single representative of family Golfingiidae was earlier reported as *Golfingia* sp. by Ghani (1996) from rocky shore of Pakistan (Paradise Point). The specimen at hand belongs to the family Themistidae of order Golfingiiformes. The genus *Themiste* includes about 10 species worldwide. Two species belonging to the genus *Themiste*, *T. (T.) hennahi* Gray, 1828 and *T. (L.) legeniformis* Baird, 1868 are known from the Indian Ocean. The species *Themiste*

(L.) lageniformis is now reported from here for the first time. The abbreviation TL is used for total length measured from anterior part (introvert) to posterior part (trunk) in mm.

Materials and methods

Specimens of Sipuncula were collected by hand in the intertidal zone at low tide and were preserved in 70% ethanol. All the material is housed in the Marine Reference collection and Resource Centre (MRC&RC).

Material examined

Sandspit, 24-04-07 (3 specimens), Lat.24° 50' 24N Long. 66° 54' 24E, TL. 41.0-69.0mm; 69.0mm. Buleji, 06-08- 96 (12 specimens), Lat.24 50 12N Long.66 49 12E .TL 18.0-34.0mm.

Systematics

Phylum: Sipuncula Stephen, 1964
Class: Sipunculidea Gibbs and Cutler 1987
Order: Golfingiiformes Cutler and Gibbs, 1987
Family: Themistidae Cutler and Gibbs 1985
Genus: *Themiste* Gray, 1828
Subgenus: *Lagenopsis* Edmonds, 1980
Species: *Themiste (Lagenopsis) lageniformis* (Baird, 1868) (Figs.1.A-E)

Results and discussion

The body of *Themiste (Langenopsis) lageniformis* is balloon shaped and pointed posteriorly; the body is smooth and opaque (Fig.1A,B). The body is 41:00 mm long and 06:00mm wide with the introvert approximately one third the trunk length. A ring of hollow ciliated tentacles surrounds the mouth. Tentacles are cunpigmented, arising from 6 primary stems that bifurcate and give rise to digitiform tentacles. The contractile vessel villi are short and branched (Fig.1D). Introvert without hooks. The papillae are uniform in size, rounded with a central pore Figure 1E.

Distribution

According to Halder (1991) this is widely distributed in temperate, subtropical, and tropical waters of the Indo-west Pacific region with a few records from the Atlantic and now for the first time recorded from northern Arabian Sea, Pakistan.

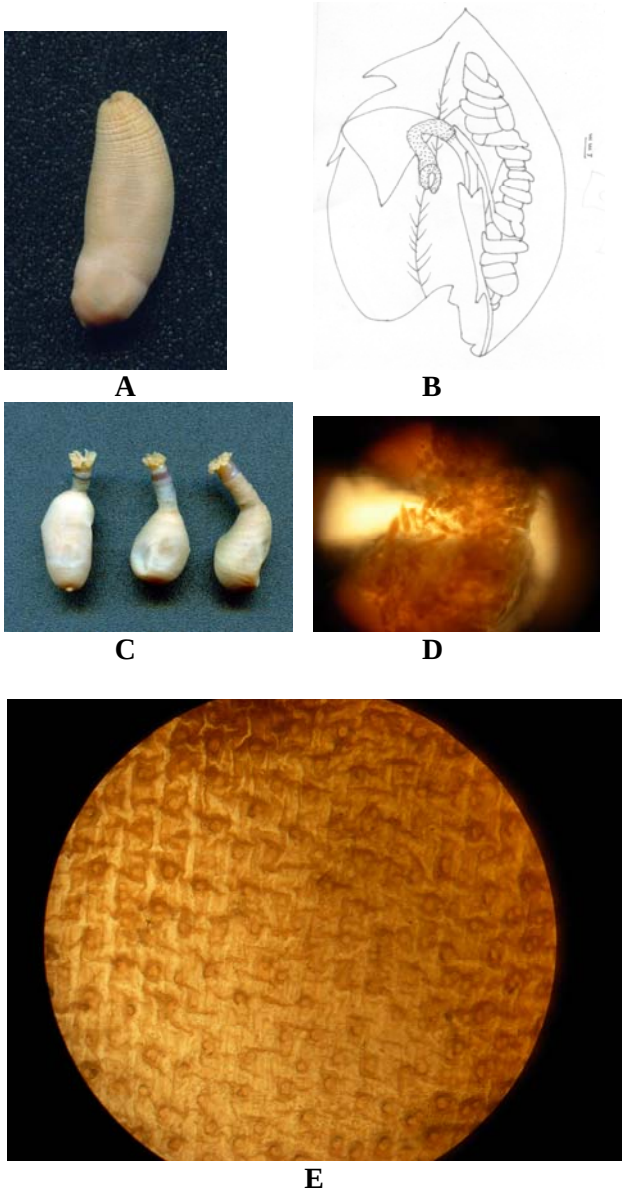

Fig. 1. *Themiste (Lagenopsis) lageniformis* (Baird, 1868); A, fully relaxed specimen; B, dissected specimen; C, with tentacular crown; D, showing branched contractile vessel villi; E, showing arrangement of papillae.

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