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NEW RECORD OF CESTODE GENUS *DILEPIS* WENILAND, 1858 IN AVIAN HOST LITTLE CORMORANT, *MICROCARBO NIGER* (AVES: PHALACROCORACIDAE) OF DISTRICT KAMBER-SHAHDADKOT, SINDH PROVINCE, PAKISTAN.

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ABSTRACT

Present study aimed to investigate Cestode fauna of avian host Little Cormorant, *Microcarbo niger* captured from Kamber-Shahdadkot district, Sindh province of Pakistan. Eight Little Cormorant *Microcarbo niger* hosts were investigated for the presence of Cestode parasites. Anesthetized birds were dissected and entire alimentary canal and associated organs were removed. A total of five specimens of Dilepidid Cestodes were recovered from the intestine of three hosts. Cestodes collected from the freshly dissected hosts were initially placed in saline solution and dehydrated in graded series of ethanol, stained with borax carmine, cleared in clove oil and xylene and finally mounted in Canada balsam permanently. Parasitic examination revealed an apparently undescribed species of genus *Dilepis* Weniland, 1858 from the intestine of the host, *M. niger*. Recovered specimens differ remarkably from other known species of genus *Dilepis* Weniland, 1858 in having some unique features such as, 12 rostellar hooks, long neck, four pre-ovarian testes, unilateral genital pore and spinose cirrus sac located at lower part of each segment. On the basis of the above morphometric variations, but absence of molecular study, present specimens are identified as *Dilepis* sp. This paper also constitutes the first record of the genus *Dilepis* Weinland, 1958 from Pakistan.

Key words: Avian Cestode; *Dilepis* Weniland, 1858; Little Cormorant *Microcarbo niger*; Sindh; Pakistan.

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INTRODUCTION

Observations made in present communication are part of the helminthological study of the Family Phalacrocoracidae from the water bodies of District Kamber-Shahdadkot, Sindh, Pakistan. Kamber-Shahdadkot district is a North-West district of Sindh province. It is an important wetland complex and composed of variety of habitats such as fresh water, agriculture land, scrubs, reed beds and fish farms. Kamber-Shahdadkot district therefore, provides suitable habitats to a variety of avian forms including resident as well as migratory birds. Moreover, water bodies are favorable habitats for definitive and intermediate hosts of different parasites. Thus, the bird under study (*M. niger*) has reasonable probability of success to become infected with Cestodes from the surrounding habitats. *Microcarbo niger* (Vieillot, 1817) is a piscivorous bird usually found in water bodies such as lakes and coastal areas. It was described as *Hydrocorax niger* in 1817 by Vieillot, later on shifted in the genus *Phalacrocorax*. Presently, some studies have placed it under the genus *Microcarbo* (Hoyo *et al.*, 2014; IUCN Redlist, 2016). Diet and feeding behavior of Little Cormorant (*M. niger*) provide fair chances to expose this bird to parasitic infections, like "helminthiasis". Amongst Helminths, Cestodes are frequent and widely distributed parasites of vertebrates generally and birds especially. Cestodes of the family Dilepididae Railliet and Henery, 1909 are widely distributed and commonly found in birds such as Spoonbills, Cormorants and Herons (Bona, 1994). Although many workers have reported cestodes from the avian hosts of Pakistan (Khan and Habibullah, 1967; Bilqees and Jehan, 1977; Bilqees, 1980, 1985; Bilqees and Fatima, 1984; Khanum *et al*., 1982; Khan *et al*., 1983; Begum *et al*., 1998; Ghazi and Bilqees, 2002; Ghazi *et al.,* 2002; Bilqees *et al*., 2003). Moreover, Akram, 1996; Dharejo *et al*., 2010; Abro *et al*., 2016 a, b, c, d, e, f, g, h and 2017 recorded different helminths of *Microcarbo niger* (Vieillot, 1817) but none of them reported Cestodes. Furthermore, the genus *Dilepis* Weinland, 1958 has not been reported from avian hosts in Pakistan. During the course of this investigation, the new record of the genus *Dilepis* Weinland, 1958 was encountered in the intestine of avian host *Microcarbo niger* (Vieillot, 1817). Hence, objective of this paper is to describe a new record of Cestode.

MATERIALS AND METHODS

In present study, a total of eight avian hosts, Little Cormorant, *Microcarbo niger* were collected (January, 2021 to April, 2022) from the study area Kamber-Shahdadkot district of Sindh province of Pakistan and investigated for the Cestode parasites. A total of five specimens of Dilepidid Cestodes were recovered from the intestine of three hosts. All specimens of Cestodes were carefully removed and processed for the preparation of permanent slides.

Cestodes collected from the freshly killed host were initially placed in saline solution, flattened by placing them between the two slides, fixed in AFA (Alcohol-Formalin-Acetic Acid), stained with Borax Carmine, dehydrated in graded series of ethanol, cleared in clove oil and xylene and finally mounted in Canada balsam. Diagrams were drawn with the help of mirror type camera Lucida and the photographs taken with Nikon digital camera. All measurements were made from the stained specimen and expressed in millimeters (mm) unless otherwise stated. Identification is done on the basis of the comparison of distinctive morphological features with those of previously described species in the literature. Keys given by Yamaguti, 1959 and other relevant literature was used for identification.

RESULTS

Morphological observations are based on five specimens of Dilepidid Cestode recovered from the intestine of three hosts.

Description (Fig. 1 & 2)**:** Body large, elongated, measuring 50-115 X 0.95-1.9 in size, consists of scolex, neck and 375 proglottids. Scolex well developed, cylindrical, measuring 0.092-1.05 X 0.95-1.09 in size, equipped with four suckers (two well developed and two rudimentary). Rostellum well developed, armed with 12 hooks arranged in double rows. Hooks of first row are larger than second row, measuring 0.106-0.120 in length. Neck long, commence after scolex, measuring 5.0-11.4 X 0.97-1.2 in size. Proglottids are wider than long. Immature proglottid 250 in number, measuring 0.10-0.156 X 0.94-1.406 in size, whereas, mature and gravid proglottids are 125 in number. Mature proglottids are filled with reproductive organs, measuring 0.25-0.466 X 0.94-1.416 in size. Gravid proglotids are 0.22-0.583 X 0.90-1.333 in size. Mature proglottid consisting of cirrus sac, vitelline gland, multilobed ovaries, four testes and uterine sac. Vitelline gland is multilobed, measuring 0.68-0.83 X 0.09-0.116 in size, situated at the base of proglottids and below ovaries. Ovaries are multilobed, present on both side of transverse area of proglottids, measuring 0.16-0.20 X 0.133-0.166 in size. Oviduct connects both ovaries. Testes are four in number, round in shape and found on both side of transverse segment. Uterus is 0.30 in length. Cirrus sac long and hairy, genital pore unilateral, well developed, situated on lower side of proglottid and protruding outside of the body. Gravid proglottid is loaded with eggs and prominent hairy cirrus sac. Eggs are 0.013-0.026 X 0.009-0.19 in size.

Taxonomic summary

Family	Dilepididae
Genus	*Dilepis* Weinland, 1958
Species	*Dilepis* sp.
Type host	Little cormorant *Microcarbo niger*
Site of infection	Intestine
Type locality	District Kamber-Shahdadkot, Sindh, Pakistan
No. of specimens recovered	05
No. of hosts examined	08
No. of hosts found positive	03

DISCUSSION

Genus *Dilepis* was created by Weinland in 1858. It contains parasites of birds and a few mammals. *D. undula* Schranks, 1788 is type of this genus collected from the hosts of family Corvidae and other birds. This species has been listed and described under the various names and its life history and presence of the larva in the earthworm was reported. The genus contains more than fifty species described from Passerine and Non-passerine birds of Europe, Asia, North America, South America, Africa and Australia (Southwell, 1921; Joyeux and Baer, 1936; Yamaguti, 1959; Swada, 1974 and Dar and Tanveer, 2013). The important features of the present recovered specimens of the genus *Dilepis* includes elongated giant body, 12 rostellar hooks arranged in double row measuring 0.106-0.120 long, neck long, four pre-ovarian testes, multilobed ovary and vitelline gland, spinose cirrus sac and unilateral genital pore, situated posterior part of the segment. While comparing with many known species of present genus *Dilepis* representing from avian hosts, the present species differs with congener in many characteristics. A large number of species of genus *Dilepis* were described from avian hosts and locality (table no.1). Present specimens have magnitude of variation in number of rostellar hooks, testes number, shapes and position of ovary and vitelline gland and position of genial pore and cirrus sac.

Present specimens have giant body and supersede in body size from already known species except *D. lepidocolpos*. Its maximum body size is 115 in length and while conger body ranges in between 100-200 in length. Present specimen is equipped with 12 rostellar hooks and can be easily figure out from other known species including *D. undula* (46-58 hooks) *D.*

rostratulae (20), *D.* y*orkei* (50 52), *D. leptophalus* (20), *D. horvathi (*52), *D. kempi* (20), *D. lepidocolpos* (20), *D. unilateralis* (20), *D. brachyarthra* (70), *D. monedulae* (40), *D. attenuta* (20), *D. cypselina* (80), *D. caprimulgina* (34), *D. globacantha* (40), *D. macrosphincter* (10), *D. urceus* (20), *D. ochropodis* (43), *D. retirostris* (20-20) and *D. limosa* (20).

The size of hooks of present specimen is larger than hooks of *D. undula*, *D. rostratulae, D. horvathi, D. lepidocolpos, D. solecina, D. unilateralis, D. brachyarthra, D. monedulae, D. attenuta, D. cypselina, D. globacantha* and *D. macrosphincter* but smaller than *D.* y*orkei, D. leptophalus* and *D. kempi*. However, it is closely resembling to *D. leptophalus, D. lepidocolpos, D. brachyarthra* and *D. urceus*.

Present specimens have large neck which differs from rest of species, whereas, *D. undula* has short necks and *D. rostratulae, D.* y*orkei*, *D. leptophalus, D. horvathi*, *D. kempi* have no neck. Moreover, neck in *D. solecina*, *D. delachauri, D. unilateralis* and rest of others is not described in literature (table no.1).

Present specimens have four pre-ovarian testes which closely resemble to *D.* y*orkei*, *D. delachauri*, *D. lepidocolpos* testes in shape and number, whereas, testes of *D. undula* (24-32 post-ovarian testes), *D. rostratulae* (40-50 post-ovarian), *D. horvathi* (15-17), *D. kempi* (3 median), *D. solecina* (numerous), *D. unilateralis* (10-12), *D. monedulae* (25), *D. macrosphincter* (numerous), *D. urceus* (3), *D. ochropodis* (50), *D. limosa* (numerous) and *D. campylancristrota* (7-8).

Present specimens have unilateral genital pore and spinose cirrus sac, situated at the posterior part of segments. It corresponds to other species except *D. campylancristrota* which has in anterior third of segment. *D. undula, D. rostratulae, D. kempi* and *D. unilateralis* have in anterior margin of segment, whereas, *D.* y*orkei, D. horvathi* possess in the middle of segment.

Present specimens have multilobed ovary which resembles *D. rostratulae*, *D. leptophalus*, *D. horvathi*, whereas, *D. undula, D. lepidocolpos*, *D. delachauri, D. unilateralis, D. monedulae, D. caprimulgina* have bilobed ovary. Vitelline gland in present species is multilobed and situated at base segment which resembles *D. lepidocolpos* but differ from *D. undula* (horse shoe shape), *D. unilateralis* (situated in middle of segment), *D. leptophalus* (situated in middle of segment).

Three species of present genus *Dilepis* have been reported from *Phalacrocorax*: *D. kempi* Southwell, 1921; *D. scolecina* Rudolphi, 1819 and *D. delachauxi* Fuhrmann, 1909. Present specimens also differ from *D. kempi, D. scolecina*, *D. delachauxi* and *D. lepidocolpos* in certain features such as size and number of hooks, number of testes, shape of ovaries, location of genital pore and cirrus sac. However, it is closely related with *D. lepidocolpos* in size of hooks, shape of genital pore, cirrus sac, and number of testes, but differs from it in number of hooks and location of genital pore and cirrus sac, therefore, cannot be assigned as *D. lepidocolpos*.

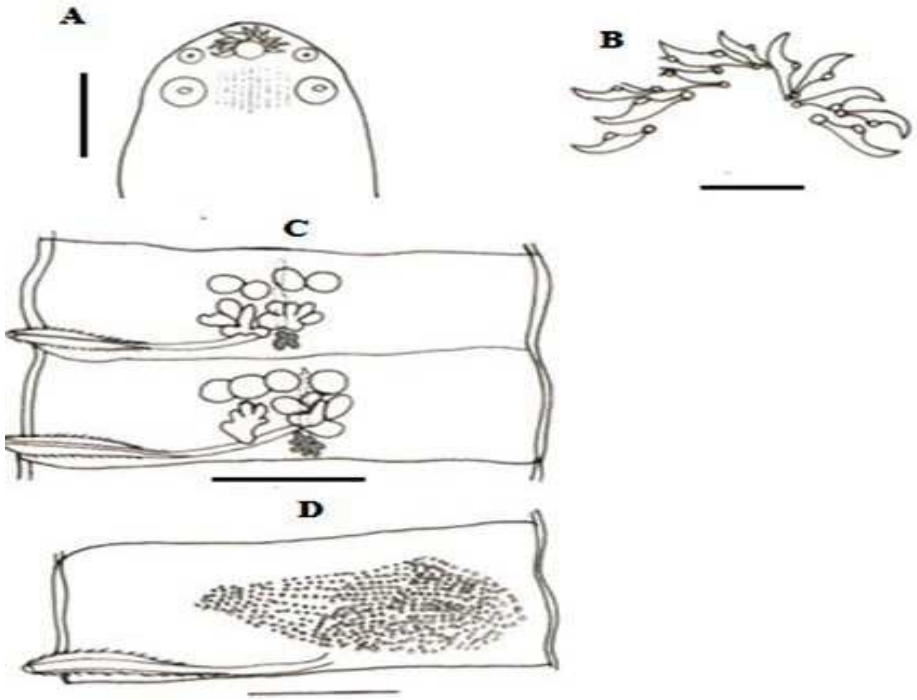

Figure No-1. *Dilepis* sp. A. Scolex; B. Hooks; C. Mature segment and D. Gravid segment.*Scale Bar:* A. 1 mm; B C & D. 0.1 mm.

It is now apparent that the present specimens of genus *Dilepis* either resemble or differ in one or more morphological features from their allied species. However, present species can be different from all other congener on the basis of some unique diagnostic morphological feature, most notably the presence of 12 rostellar hooks in double row, 4 pre-ovarian testes, long neck, unilateral genital pore and spinose cirrus sac at lower edge of each segment, multilobed ovary as well as vitelline gland and their (ovary and vitelline gland) location in each segment. These unique features certainly fulfil the required morphometric criterion to designate it as new species. However, molecular analysis is essential for erecting new species. Therefore, present specimens are identified as *Dilepis* sp. The genus *Dilepis* Weinland, 1958 is being reported for the first time from Pakistan.

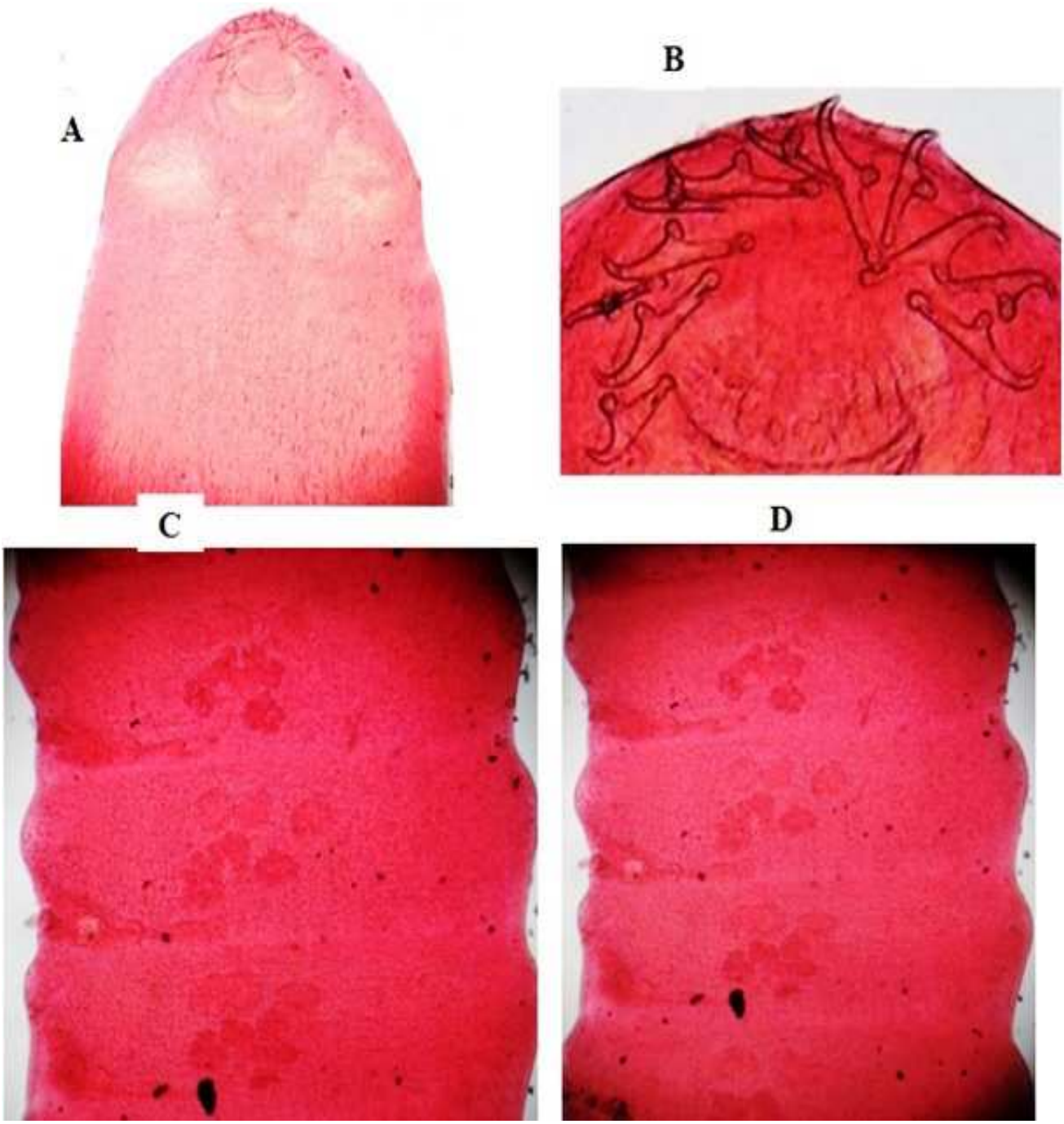

Figure No-2. *Dilepis* sp. A. Scolex; B. Hooks; C & D. Mature segment

Table No-1. Species of genus *Dilepis* with host and locality

Sr. no.	Name of species	Name of host	Locality	Reference
1	*D. ardea* Rausch, 1935	*Ardea herodias*	America (USA)	Yamaguti, 1959
2	*D. ardeolae* Singh, 1952	*Ardeola grayi*	Asia (India)	Yamaguti, 1959
3	*D. attenuata* (Duj., 1845)	*Anthus pratensis, Troglydytes, Fringilla coelebes, Passer domesticus*, *P. montanus*	Europe	Yamaguti, 1959
4	*D. australiensis* Johnston, 1911	*Himantopus leucocephalus*	Australia	Yamaguti, 1959
5	*D. bancrofti* Johnston, 1912	*Platycerus eximius*	Australia	Yamaguti, 1959
6	*D. bicoronata* Fuhrmann, 1908	*Mesembrinibis cayennensis*	South America	Yamaguti, 1959
7	*D. brachyarthra* Choldk, 1906	*Corvus cornix, Turdus merula*	Europe	Yamaguti, 1959
8	*D. campylancristrota* (Wedl, 1855)	*Ardea cinerea*, *Ardeola grayi, Herodias garzetta*	Europe, Asia (India)	Yamaguti, 1959
9	*D. capellae* Yamaguti, 1935	*Capella solitaria*, *Rostratula benghalensis*	Asia (Japan)	Yamaguti, 1959
10	*D. caprimulgina* Neslobinsky, 1911	*Caprimulgus europaeus*	Europe (Russia)	Yamaguti, 1959
11	*D. crassirostrata* Fuhrmann, 1908	*Tigrisoma brasiliensis*	America	Yamaguti, 1959
12	*D. cypselina* Neslobinsky, 1911	*Cypselus apus*, *Dendrocitta leucogaster*	Europe (Russia), Asia (India)	Yamaguti, 1959; Joyeux & Baer, 1936
13	*D. distincta* (Lonnberg, 1889)	*Larus canus*	---	Yamaguti, 1959
14	*D. fotedari* Chishti, 1980	*Anas platyrhynchos*	Kashmir	Chishti, 1980
15	*D. fovea* Meggitt, 1933	*Dendrocitta rufa*	Asia (India)	Yamaguti, 1959
16	*D. fuhrmani* Raillet & Henrry, 1909	*Hoploxypterus cayanus*	South America (Brazil)	Yamaguti, 1959
17	*D. globacanth* Fuhrmann, 1913	*Caprimulgus europaeus*	Europe	Joyeux & Baer, 1936
18	*D. glareola*	*Tringa glareola*	Europe (Russia)	Yamaguti, 1959
19	*D. hilli* Polk, 1904	*Florida caerulea*	America (USA)	Polk, 1941; Yamaguti, 1959
20	*D. hoplites* (Linstow, 1903)	*Ardea*	Siberia	Yamaguti, 1959
21	*D. horvathi* Kotlan, 1923	*Megapodius brunneiventris*	Australia (New Guinea)	Yamaguti, 1959
22	*D. irregularis* Southwell et Lake, 1930	*Rostratula benghalensis*	Africa (Belgian Congo)	Yamaguti, 1959
23	*D. jacobsni* Baylis, 1929	*Eucichla cyanura*	Asia (Indonesia)	Yamaguti, 1959
24	*D. kempi* Sowthwell, 1921	*Phalacrocorax pygmaeus*	Asia (India)	Southwell, 1921
25	*D. lepidocolpos*	*Phalacrocorax niger*	Asia (Srilanka)	Burt, 1936
26	*D. leptophallus* Kotlan, 1922	*Megapodius brunneirventris*	Australia (New Guinea)	Yamaguti, 1959
27	*D. limosa* Fuhrmann, 1907	*Limosa limosa*, *Numenius phaeopus, Tringa ochropus*	Africa (Egypt) Europe	Yamaguti, 1959; Joyeux & Baer, 1936
28	*D. macrocephala*	*Psophia crepitans*	South America (Brazil)	Yamaguti, 1959
29	*D. macroephinctor* Fuhrmann, 1909	*Ardeola ralloides, Ardea purpurea*	Africa, Asia (India), Europe	Yamaguti, 1959; Joyeux & Baer, 1936
30	*D. maxima* Goss, 1941	*Microcarbo melanoleucus*	Australia	Yamaguti, 1959
31	*D. megacirrosa* Ortlepp, 1940	*Chrysochloris asiatica*	Cape province, South Africa	Yamaguti, 1959

32	*D. megalorhyncha* (Krabbe, 1869)	*Erolia maritima*	Europe (Greenland)	Yamaguti, 1959
33	*D. monedula* Neslobinsky, 1911	*Corvus monedula*	Europe (Russia)	Yamaguti, 1959; Joyeux & Baer, 1936
34	*D. nasuta* Fuhrmann, 1908	*Theristicus melanopsis*	South America (Brazil)	Yamaguti, 1959
35	*D. nymphoides* Clerc, 1903	*Erolia minuta, E. subminuta*	Europe (Russia)	Yamaguti, 1959
36	*D. ochropodis* Neslobinsky, 1911	*Totanus ochropus*, *Tringa oellropus*	Europe (Russia)	Yamaguti, 1959; Joyeux & Baer, 1936
37	*D. odhneri* Fuhrmann, 1909	*Oedicnemus senegalensis*, *Burhinus capensisaffinis*	Africa	Yamaguti, 1959
38	*D. oligorchida* Fuhrmann, 1906	*Busarellus nigricolliss*	South America (Brazil)	Yamaguti, 1959
39	*D. orientalis* Yamaguti, 1959	*Turdus aurues*	Asia (Japan)	Yamaguti, 1959
40	*D. papillifera* Fuhrmann, 1908	*Florida caerulea*	South America (Brazil)	Yamaguti, 1959
41	*D. retirostris* Krabbe, 1869	*Erolia minuta*	Europe	Joyeux & Baer, 1936
42	*D. rostratulae* Swada & Kifune, 1947	*Rostratula bengalensis*	Asia (Japan)	Swada & Kifune, 1947
43	*D. sobolevi* Spassky, 1946	*Luscinia luscinia*	Europe (Russia)	Yamaguti, 1959
44	*D. transfuga* (Krabbe, 1869)	*Platelea ajaja*	America	Yamaguti, 1959
45	*D. tringae* Cholodk, 1912	*Platyrhncha, Limicola falcinellus*	Europe	Yamaguti, 1959
46	*D. turdi* Yamaguti, 1935	*Turdus aurues*	Asia (Japan)	Yamaguti, 1959
47	*D. unilateralis* (Rud., 1819)	*Herodia egretta, Ardea cinerea, Ardeola grayi, Butorides virescens*, *Garzetta garzetta*	Europe	Yamaguti, 1959
48	*D. undula* Schrank, 1788	*Corvus monedula*, *C. splendens*, *C. macrorhychos*, *Ardea cinerae, A. puperae*	Asia (Kashmir), Europe	Dar & Tanveer, 2013; Illescas-Gomez, 1981; Yamaguti, 1959
49	*D. urceus* Joyeux & Baer, 1936	*Patalea leucorodia*	Europe	Joyeux & Baer, 1936
50	*D. yorkei* Kotlan, 1922	*Megapodius brunneiventris*	Australia (New Guinea)	Kotlan, 1922

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