MORPHOMETRIC DISCRIMINANT ANALYSIS OF THREE SPECIES OF *PETASIGER* DIETZ, 1909 (DIGENEA: ECHINOSTOMATIDAE)

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Summary :

The morphology of three Palaearctic species of the genus *Petasiger* Dietz, 1909, parasitizing grebes and exhibiting a significant degree of overlap, was studied. Ninety-three specimens belonging to *P. grandivesicularis, P. neocomense* and *P. pungens* were compared using univariate and multivariate statistical analyses of 17 metrical characters. A stepwise discriminant analysis run in three variants and based on seven variables (body length, body width, oral sucker diameter, pharynx diameter, ventral sucker diameter, posterior testis diameter and oesophagus length) yielded a 100 % accurate classification. The first canonical discriminant function separated specimens belonging to *P. grandivesicularis* and *P. pungens,* and the second function effectively differentiated the *P. neocomense* sample. Two variables (pharynx diameter and oesophagus length) were strongly correlated with the canonical functions and, when used alone, discriminated the three *Petasiger* spp. A simple key based on two variables used in the discriminant analyses and a table of the qualitative and quantitative characters used for the identification of the three species are presented.

KEY WORDS : Digenea, Echinostomatidae, *Petasiger, Petasiger grandivesicularis, Petasiger neocomense, Petasiger pungens,* morphology, variability, multivariate analysis.

Résumé : ANALYSE MORPHOMÉTRIQUE DISCRIMINANTE DE TROIS ESPÈCES DE *PETASIGER* DIETZ, 1909 (DIGENEA : ECHINOSTOMATIDAE)

La morphométrie de trois espèces voisines paléarctiques du genre Petasiger *Dietz, 1909 parasitant les grèbes est étudiée. Quatre-vingt treize spécimens adultes de* P. grandivesicularis, P. neocomense *et* P. pungens *ont été comparés à l'aide d'analyses unifactorielles et multifactorielles de 17 paramètres morphométriques. L'analyse discriminante, exécutée en trois variantes et basée sur sept variables (longueur et largeur du corps, diamètres de la ventouse orale, du pharynx, de la ventouse ventrale et du testicule postérieur, et longueur de l'œsophage), a permis une identification 100 % exacte. La première fonction canonique discriminante a différencié les spécimens de* P. grandivesicularis *et* P. pungens *tandis que la deuxième fonction différencie le groupe* P. neocomense *des deux autres. Deux variables (diamètre du pharynx et longueur de l'œsophage) présentent une grande corrélation avec les fonctions canoniques et à elles seules permettent d'identifier les trois espèces de* Petasiger. *Une clé construite à partir de ces deux variables identifées par l'analyse discriminante ainsi qu'un tableau décrivant les caractères qualitatifs et quantitatifs utilisés pour l'identification des trois espèces sont proposés.*

MOTS CLÉS : *Digenea, Echinostomatidae,* Petasiger, Petasiger grandivesicularis, Petasiger neocomense, Petasiger pungens, *morphologie, variabilité, analyse multifactorielle.*

INTRODUCTION

Since the erection of the genus *Petasiger* by Dietz (1909) for a new species, *P. exaeretus* Dietz, 1909, from cormorants, 13 species of *Petasiger* have been described from grebes (Aves: Podicipedidae). Of these, six species [*P. pungens* (Linstow, 1894), *P. megacanthum* (Kotlan, 1922), *P. neocomense* Fuhrmann, 1927, *P. lobatus* Yamaguti, 1933, *P. grandivesicularis* Ishii, 1935, and *P. brevicauda* (Ishii, 1935)] were recorded within the Palaearctic, six species (*P. nitidus* Linton, 1928, *P. novemdecim* Lutz, 1928, *P. chandleri* Abdel-Malek, 1952, *P. floridus* Premvati, 1968, *P. pseudoneocomense* Bravo-Hollis, 1969, and *P. caribbensis* Nassi, 1980) were described from the New World and one (*P. australis* Johnston and Angel, 1941) occurs in Australia.

Although the generic status of these species has been questioned (see Prudhoe, 1945; Odening, 1962), they all appear to constitute a natural group of grebe-specific parasites, all possessing 19 collar spines. However, the members of this flock of closely related species exhibit a significant degree of overlap in the morphometric characters, which, added to the lack of reliable qualitative differential features, has lead in many cases to taxonomic uncertainties (see Prudhoe, 1945; Skrjabin & Bashkirova, 1956; Odening, 1962; Nasir *et al.*, 1972; Kostadinova & Gibson, in press). As no study of the intraspecific variability of adult morphology of the 19 collar-spined *Petasiger* species complex has been undertaken, a detailed analysis of

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metrical features of the three most common Palaearctic species, *P. grandivesicularis*, *P. neocomense* and *P. pungens*, is presented here. In addition, since only sample ranges for measurements have previously been presented for all documented records of *Petasiger* spp., an attempt will be made to test statistically differences in the metrical features of the three species using newly-collected materials in order to elucidate those which are the most discriminating.

MATERIALS AND METHODS

Parasite materials

Four sets of adult worms were examined: one set from Japan and three sets from Bulgaria. The latter included 21 specimens of *P. grandivesicularis* and 20 specimens of *P. pungens* from the small intestine of *Tachybaptus ruficollis*, and 24 specimens of *P. neocomense* from the small intestine of *Podiceps cristatus*. Birds were collected on the Bulgarian Black Sea coast (Lake Durankulak). The Japanese set included 28 voucher specimens of *P. grandivesicularis* from the small intestine of *T. ruficollis japonicus* from Lake Ogura, Japan, originally described by Yamaguti (1939).

All of the Bulgarian material was collected live, killed and fixed in hot water, preserved in 70 % alcohol, stained with iron-acetocarmine, which also acts as a fixative (Georgiev *et al.*, 1986), and mounted in Canada balsam (AK collection Nos 10121, 14626, 10065, 11156). The Japanese specimens deposited in the Meguro Parasitological Museum (No. 22609, SY7001) have been fixed in acetic sublimate under a cover glass, stained with Heidenhain's hematoxylin and mounted in balsam. Measurements are given in micrometres.

The morphology of our material agreed with the original descriptions and also corresponded well with the subsequent redescriptions of the three species under study (Prudhoe, 1945; Macko, 1959; Odening, 1962, 1965; Borgarenko, 1984; Chen *et al.*, 1985).

Statistical analyses

The following metrical features (variables) were subjected to analyses (see Fig. 1 and Table I) :

1. Body length (BL); 2. Maximum body width (BW); 3. Head collar width (CW); 4. Angle spines length (ASL); 5. Dorsal spines length (DSL); 6. Oral sucker diameter (DOS); 7. Prepharynx length (PL); 8. Pharynx diameter (DPH); 9. Oesophagus length (OL); 10. Cirrus-sac length (CSL); 11. Maximum cirrus-sac width (CSW); 12. Ventral sucker diameter (DVS); 13. Anterior testis diameter (DAT); 14. Posterior testis diameter (DPT); 15. Ovary diameter (DOV); 16. Egg length (EL); 17. Egg width (EW).

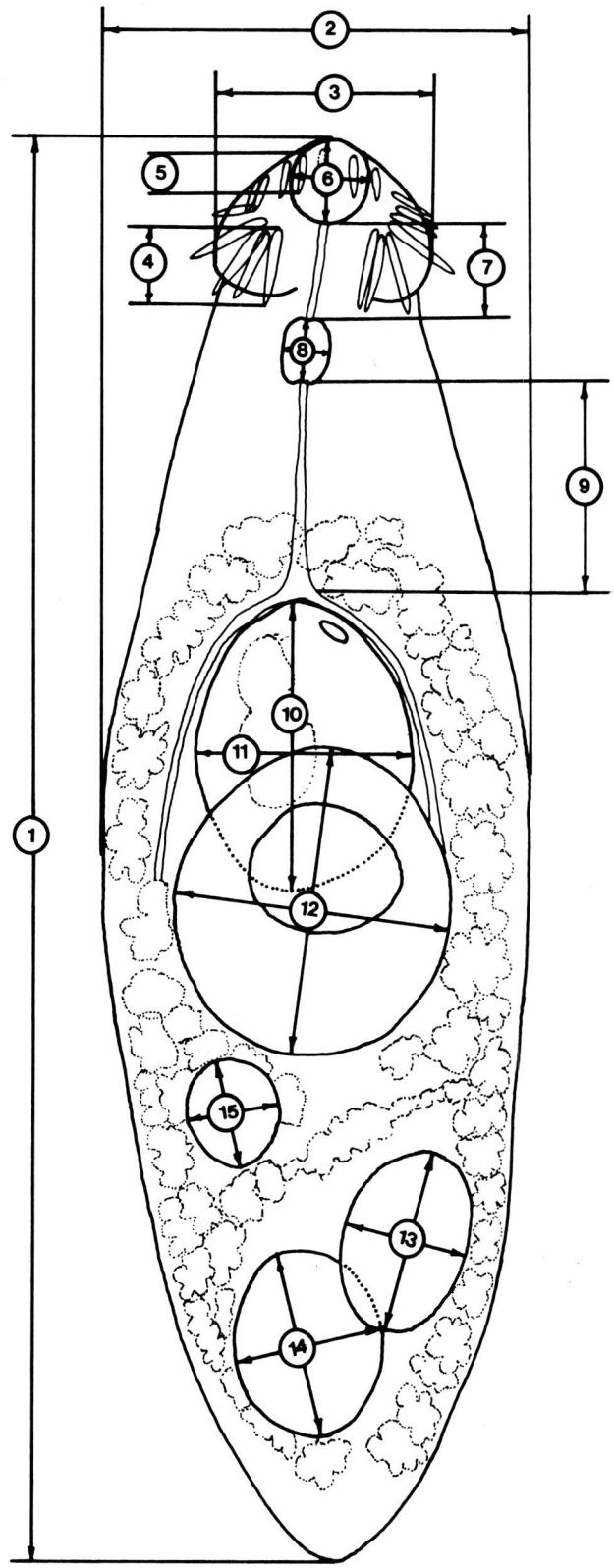

Fig. 1 — Schematic illustration of *Petasiger grandivesicularis*, showing the metrical features used as variables in the analyses.

Species	*P. grandivesicularis*			*P. neocomense*			*P. pungens*		
No. Variable	*n*	Mean	Lo-Up CLs	*n*	Mean	Lo-Up CLs	*n*	Mean	Lo-Up CL
1. BL	49	1,005	973-1,040	24	1,349	1,277-1,425	20	1,528	1,426-1,637
2. BW	48	320	304-337	23	395	367-426	20	478	457-500
3. CW	46	155	149-162	22	216	199-235	17	207	198-217
4. ASL	199	69	67-70	46	85	83-88	18	78	73-84
5. DSL	83	40	39-41	47	66	63-69	12	49	45-53
6. DOS	49	60	58-62	24	82	78-87	20	101	98-104
7. PL	47	53	49-57	23	54	46-64	15	45	32-64
8. DPH	48	45	44-46	24	63	60-65	19	81	78-84
9. OL	45	130	124-138	24	275	250-303	16	138	117-163
10. CSL	32	157	145-170	20	192	178-207	5	186	177-196
11. CSW	37	142	126-161	21	105	95-116	3	130	117-143
12. DVS	49	216	207-226	24	270	252-290	20	256	247-264
13. DAT	34	136	129-142	24	149	138-160	18	189	179-201
14. DPT	46	128	122-135	24	140	130-150	20	197	182-214
15. DOV	19	75	67-85	17	67	59-74	16	78	72-85
16. EL	28	78	76-81	25	71	68-74	19	83	80-87
17. EW	28	49	47-52	25	43	41-45	19	46	43-49

Table I. — Means and their confidence intervals (Lo-Up 95 % confidence limits) for variables under comparison.

Approximate diameters (for Nos 6, 8, 12, 13, 14 and 15) were calculated as: (length + width)/2. Prior to analysis the data were log-transformed ($\log_{10}$). Firstly, the descriptive univariate statistics [mean values, standard deviations and 95 % confidence limits (CLs)] for all of the variables were calculated for every species. Then one-way analyses of variance (ANOVA) were carried out to test the equality of the species means for each variable (Sokal & Rohlf, 1981).

A set of variables selected at the second step was submitted to discriminant analysis using the species as a grouping variable. The forward stepwise variable selection procedure was applied using the minimization of Wilks' lambda as a selection criterion. Specimens with a missing value for most of the variables were not included in the canonical analysis: these were two specimens from the Bulgarian set and four specimens from the Japanese set of *P. grandivesicularis*; four *P. pungens*; and one *P. neocomense*. Since cases with missing information for any of the predictor variables were excluded from the analyses, some variables showing high numbers of missing values were eliminated in order to increase the sample size. As a result, seven variables (BL, BW, DOS, DPH, DVS, DPT and OL) and 82 specimens were used.

Since *i)* the measurements of the Japanese set of *P. grandivesicularis* (described by Yamaguti, 1939) varied outside the limits given by Ishii (1935) and *ii)* the univariate statistical tests revealed significant differences between the two samples of this species (Bulgarian and Japanese) in 13 of the 17 metrical features ($P < 0.05$; data not shown), the analysis was run in three variants. Canonical variates from distance measurements for each species were computed using: *i)* data from the pooled *P. grandivesicularis* set [82 cases (43 *P. grandivesicularis*, 23 *P. neocomense* and 16 *P. pungens*)]; *ii)* data from the Japanese subset of specimens of *P. grandivesicularis* [63 cases (24 *P. grandivesicularis*, 23 *P. neocomense* and 16 *P. pungens*)]; *iii)* data from the Bulgarian subset of specimens of *P. grandivesicularis* [58 cases (19 *P. grandivesicularis*, 23 *P. neocomense* and 16 *P. pungens*)].

After the variables considered to be the most discriminating had been chosen, a simple classification key was prepared. The threshold values used in the key were calculated as described by Di Deco *et al.* (1994).

All statistical analyses were performed using SPSS/PC+ (Norusis, 1990) at the Hungarian Natural History Museum.

RESULTS

The means of the metrical features of *P. grandivesicularis* (the two sets pooled), *P. neocomense* and *P. pungens* and their confidence intervals are given in Table I. In order to estimate which of the 17 metrical features might be useful for the identification of the three *Petasiger* species, their means and 95 % confidence limits were examined for overlap between the three sets. Five variables (BL, BW, DSL, DOS and DPH) showed no overlap of any kind. Four other characters (CW, ASL, CSL and DVS), although having close mean values and overlapping confidence intervals for *P. neocomense* and *P. pungens* sets, separated the *P. grandivesicularis* set.

Differences in measurements of the variables were tested statistically. The one-way analysis of variance revealed highly significant differences in the means of 15 of the 17 variables among the three species (Table II).

No. Variable	df	F	P	Homogeneous groups*
1. BL	92	88.28	***	–
2. BW	90	45.06	***	–
3. CW	84	44.96	***	(2 + 3)**
4. ASL	262	65.62	***	–
5. DSL	141	170.55	***	–
6. DOS	92	194.39	***	–
7. PL	–	–	–	–
8. DPH	90	359.72	***	–
9. OL	84	109.91	***	(1 + 3)
10. CSL	56	7.56	***	(2+3) + (3+1)
11. CSW	–	–	–	-
12. DVS	92	22.01	***	(2 + 3)
13. DAT	75	28.25	***	(2 + 1)
14. DPT	89	42.18	***	(2 + 1)
15. DOV	–	–	–	–
16. EL	71	21.09	***	–
17. EW	71	10.94	***	(1+3) + (3+2)

* Groups in which the means are not significantly different from one another (Tukey test, rejection level 0.05).
** 1 = *P. grandivesicularis* set; 2 = *P. neocomense* set; 3 = *P. pungens* set.
*** P < 0.001; – P > 0.05.

Table II. — Significance of variation among the three species of *Petasiger* as shown by the ANOVA results.

In general, with the exception of the width of cirrus-sac and egg size, *P. grandivesicularis* exhibited the smallest dimensions of the metrical features compared. *P. neocomense* had the longest angle and dorsal spines, longest oesophagus and largest ventral sucker. The *P. pungens* set was characterized by the largest body, oral sucker, pharynx, testes and ovary. These results indicate that there are enough grounds for the three *Petasiger* species under study to be distinguished on the basis of the metrical variables discussed above. Consequently, the characters presented in Table III are proffered as being useful for the discrimination of the three commonest Palaearctic species of *Petasiger*. This also includes, in addition to the metric variables commented upon above, some qualitative features which were re-examined during the course of the study.

A stepwise discriminant analysis run to examine which variables best separate the three species sets revealed a combination of seven variables (BL, BW, DOS, DPH, DVS, DPT and OL) which yielded a 100 % correct identification of the three taxa in all three variants (see *Materials and Methods* for details).

Both canonical discriminant functions (CDFs) had small values of Wilks' lambda (0.015 and 0.19; 0.009 and 0.199; and 0.007 and 0.146 for the three analyses, respectively) and were significant (Chi-squared approximation, $p < 0.0001$) in all three variants. The first function accounted for 74.5, 77.9 and 84.2 % of the total between-species variability in the first, second and third variants, respectively, while the second accounted for the remaining 25.5, 15.8 and 22.1 % of the variability, respectively. As illustrated in Fig. 2 A, the first CDF clearly separated specimens belonging to *P. grandivesicularis* and *P. pungens*, and the second CDF effectively differentiated the *P. neocomense* sample in all three variants.

Comparative data on the factor structure matrix (Table IV) showed that two variables (DPH and OL) were the most important features for discriminating the three *Petasiger* species along CDF1 and CDF2, respectively. These variables also exhibited the lowest correlation with the other predictor variables (OL, range 0.20-0.47; DPH, range 0.28-0.47), and subsequent analysis showed that a combination of DPH and OL alone was sufficient to discriminate the three *Petasiger* species (100 % accurate classification; see also the ordination pattern in Fig. 2 B).

Finally, a simple classification key, in which the actual values for pharynx width were used rather than the calculated diameters, was constructed. The means and standard deviations of the oesophageal length (OL) for *P. grandivesicularis* and *P. pungens* (pooled) (135.9 ± 30.9) were used to obtain the threshold value for the first step. Then the threshold value of the pharynx width (PHW) between *P. pungens* and *P. grandivesicularis* was calculated for the second step. These steps resulted in the following key:

(1) OL > 185 µm ..... *P. neocomense*
< 185 µm ..... (2)
(2) PHW > 52 µm ..... *P. pungens*
< 52 µm ..... *P. grandivesicularis*

DISCUSSION

There has been considerable comment in the literature on the morphological criteria used for distinguishing the 19-collar spined forms of *Petasiger* at the specific level, as well as whether groupings at the subgeneric level exist (Bashkirova, 1941; Mendheim, 1943; Skrjabin & Bashkirova, 1956; Yamaguti, 1958). Prudhoe (1945) re-described « *Petasiger* » *pungens* and « *P.* » *megacanthus*, which he provisionally assigned to *Petasiger* and regarded *P. neocomense* and *P. nitidus* as synonyms of « *P.* » *megacanthus*. This author suggested that *P. lobatus*, *P. grandivesicularis* and *P. australis* bear a strong resemblance to « *P.* » *pun-*

Character	*P. grandivesicularis*	*P. neocomense*	*P. pungens*
Body shape	fusiform; widest anterior to ventral sucker	fusiform; widest at level of ventral sucker	elongate-oval; widest posterior to ventral sucker
Cirrus	bulb-like	long, tubular	bulb-like
Pars prostatica	well developed	not developed	intermediate
Vitellarium	large follicles forming two fields fusing at intestinal bifurcation and posteriorly to ventral sucker and testes	small follicles in two non-fusing fields	large follicles in two fields fusing posterior to testes
Testes	oblique	oblique	tandem, rarely oblique
Body and organ size	smallest	intermediate	largest
Collar spines	smallest	largest	intermediate
Angle spines (CLs)	67-70	83-88	73-84
Dorsal spines (CLs)	39-41	63-69	45-53
Eggs	intermediate	smallest	largest
Length (CLs)	76-81	68-74	80-87
Width (CLs)	47-52	41-45	43-49
Metrical features (in relation to body length):			
Body width	intermediate	smallest	largest
Collar width	intermediate	largest	smallest
Prepharynx length	largest	intermediate	smallest
Oesophagus length	intermediate	largest	smallest
Ventral sucker diam.	intermediate	largest	smallest
Posterior testis diam.	smallest	intermediate	largest

Table III. — Characters useful for distinguishing the three Palaearctic species of *Petasiger*.

gens. Nasir *et al.* (1972) went further, placing in synonymy with *P. pungens* the following: *P. australis*, *P. chandleri*, *P. brevicauda*, *P. floridus*, *P. grandivesicularis*, *P. lobatus*, *P. longicirratus* Ku, 1938, « *P. parvispinosus* » [sic] and *P. spasskyi* Oschmarin, 1974. Assuming that both metrical and qualitative features previously used to separate the adult parasites are subject to intraspecific variation, these authors suggested retaining the above species as conspecific unless life-history studies were able to furnish evidence to the contrary.

Obviously, further work is needed on both the life-history and adult morphology of *Petasiger* spp. Nevertheless, at the present time, adult morphology is all we have in most cases to provide criteria for erecting new species within the genus *Petasiger*, for the identification of Palaearctic material and for solving taxonomic problems, since life-history data for the Palaearctic species are almost non-existent (Karmanova, 1971; Kostadinova & Chipev, 1992). Unfortunately, current knowledge of the morphology of the Palaearctic species of *Petasiger* does not permit reliable species identification, since existing morphometric data for the three species under study exhibit overlapping ranges for all characters with the exception of the cirrus-sac length (see Fuhrmann, 1927; Ishii, 1935; Yamaguti, 1939; Mendheim, 1940; in Skrjabin & Bashkirova, 1956; Prudhoe, 1945; Skrjabin & Bashkirova, 1956; Macko, 1959; Odening, 1962; Odening, 1965; Cankovic *et al.*, 1983; Borgarenko, 1984; Chen *et al.*, 1985; Iskova, 1985).

The results of the univariate and multivariate statistical analyses presented here, although carried out on a restricted set of characters and specimens, demonstrate that reliable morphometric differences supporting the distinct status of the compared species do exist. Our data reveal a number of variables (BL, BW, ASL, DSL, DOS, DPH, DVS, DPT, OL and EL) that exhibit important morphometric differences between the three species. Although the material did not permit the acquisition of sufficient data on the measurements of spines and eggs for each specimen to include these variables

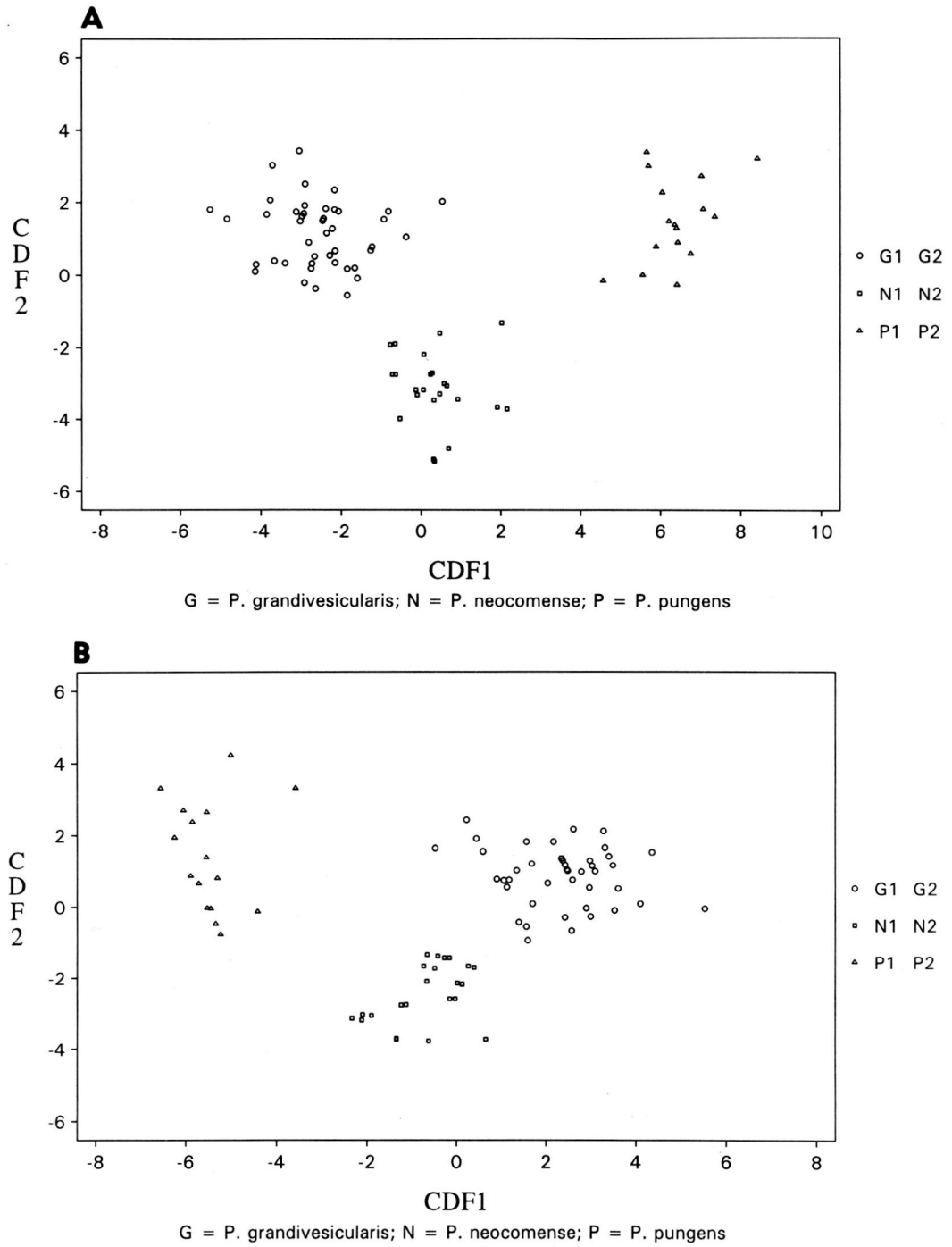

Fig. 2. — Scatterplot of the discriminant scores of specimens belonging to the three species of *Petasiger*. A. Canonical discriminant functions CDF1 and CDF2 derived using 82 specimens and 6 variables (Variant 1). B. Canonical discriminant functions CDF1 and CDF2 derived using 85 specimens and 2 variables (DPH and OL).

	Variant 1 (n = 82)		**Variant 2** (n = 63)		**Variant 3** (n = 58)	
Variable	CDF1	CDF2	CDF1	CDF2	CDF1	CDF2
DPH	0.805*	– 0.404	0.580*	– 0.509	0.623*	0.453
OL	0.054	– 0.808*	– 0.045	– 0.804*	– 0.051	0.696*
DPT	0.252	0.066	0.179	0.124	0.254	0.035
DVS	0.131	– 0.261	0.034	– 0.222	0.129	0.356
BL	0.361	– 0.290	0.215	– 0.269	0.306	0.382
BW	0.269	– 0.105	– **	–	0.296	0.263
DOS	–	–	0.379	– 0.315	0.470	0.416

** Variable excluded from the model.

Table IV. — Comparative data on the pooled-within groups correlations between the variables and the canonical discriminant functions (CDFs). The values indicating the highest correlation of a variable with the CDF are marked with an asterisk.

in the discriminant analysis, the univariate tests suggest that these features also contribute to the discrimination of the three taxa. However, the variability of some characters, as indicated by their CLs and CDF scores, suggest the need for detailed studies on the inter-populational variability within *Petasiger* spp. All these considerations point to the desirability of a thorough review of the genus *Petasiger* with re-descriptions of the valid species.

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