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High taxonomic resolution as a determinant on finding new species and new records in the Río de La Plata basin: a case on Chydoridae (Crustacea: Branchiopoda: Cladocera)

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ABSTRACT - Recent worldwide taxonomical research on Cladocera is discovering a number of new species but hardly in South America. The Río de La Plata is the second largest basin in the continent and the knowledge on cladocerans of the different sub-basins is uneven. The recent increase in the number of cladocerans species worldwide inspired this survey in the Río de La Plata basin. It was expected that new species, or at least a significant number of new records, could be found which would contribute to the richness of the least studied sub-basins. Twenty three sampling locations were selected and the Cladocera were analyzed in detail to the species level. The family Chydoridae was targeted due to its high species richness. Fifty species of Chydoridae were recorded, two of them probably new for science and a third one whose taxonomical status could not be further addressed, due to insufficient material for analysis. All sub-basins had at least one specimen of one or both new species. The contribution of new species was more homogeneous among sub-basins when compared to the total contribution of new registers. It was concluded that the improvement of the taxonomical resolution and extensive samplings are fundamental in assessing the real cladoceran richness in the basin and that knowledge based on just a few research centers is not enough to promote secure taxonomical and biogeographical considerations yet.

Key words: Phytophilous cladocerans, Geographical distribution, Taxonomy, Biogeography

Introduction

Research on cladoceran taxonomy is being carried out all around the world. The high taxonomical resolution used in the researches has resulted in a continuous increase in the number of known species and reallocation of former taxa into new groups (e.g. Van Damme and Dumont, 2010; Van Damme *et al.*, 2011).

Cladocera investigation in South America and some other continents (Chatterjee *et al.*, 2013) has historically focused on the ecology of the group, rather than on taxonomical investigation. Due to this bias, contemporary research that applies a higher level of resolution for the taxa identification is frustrating as there are inconsistencies and many unidentified species.

There is not only a historical lack of taxonomical knowledge, but also a marked heterogeneity in the geographical distribution of the studies, which are concentrated around scientific centers. Many of these centers are in the Río de La Plata basin, the second largest basin in South America and densely populated. In this basin, it is possible to find relatively well studied regions, such as the middle Paraná River sub-basin (e.g. José de Paggi and Paggi, 2007), but also almost unstudied regions as the Paraguay River sub-basin, directly connected to the Pantanal, an extensive wetland-floodplain system.

The worldwide increasing number of species and constant reallocation of taxa and the regional lack of taxonomical studies created a knowledge

vacuum on Cladocera in the Río de La Plata basin and inspired a comprehensive survey.

In this paper, only the family Chydoridae Stebbing, 1902 was addressed due to their likely high species richness. Additionally, this family is also subject of recent taxonomical studies which provides a large amount of new information to be considered in the identification process.

Based on the above cited geographical organization of knowledge in the basin, it was expected to find a higher proportion of new species and new records in less-studied regions of the basin.

Material and Methods

Cladocerans were sampled with plankton net (68 μm), in 23 macrophyte-rich sites of the Río de La Plata basin during summer and winter 2010 (Fig. 1). Table 1 lists the sampling sites by region, their coordinates and level of knowledge evaluation.

The identification of Chydoridae was performed on the basis of exhaustive morphological analyses, at species level, based on specialized literature (e.g. Smirnov, 1996; Elmoor-Loureiro, 1997; Van Damme and Dumont, 2008; Van Damme *et al.*, 2011).

The relative contribution of new taxa and new records were represented by their proportion in the total number of species sampled in each region.

Results

A total of 50 species of Chydoridae was found in this study (Tab. 2).

Two possible new species of Chydoridae are proposed, both presently being described. The first species belongs to *Alona* Baird, 1843 *sensu lato*, which include the *Alona*-like species that cannot be placed into true genus *Alona* (Van Damme and Dumont, 2008) (Fig. 2). The second species (Fig. 3) shared many features with *Anthalona simplex*

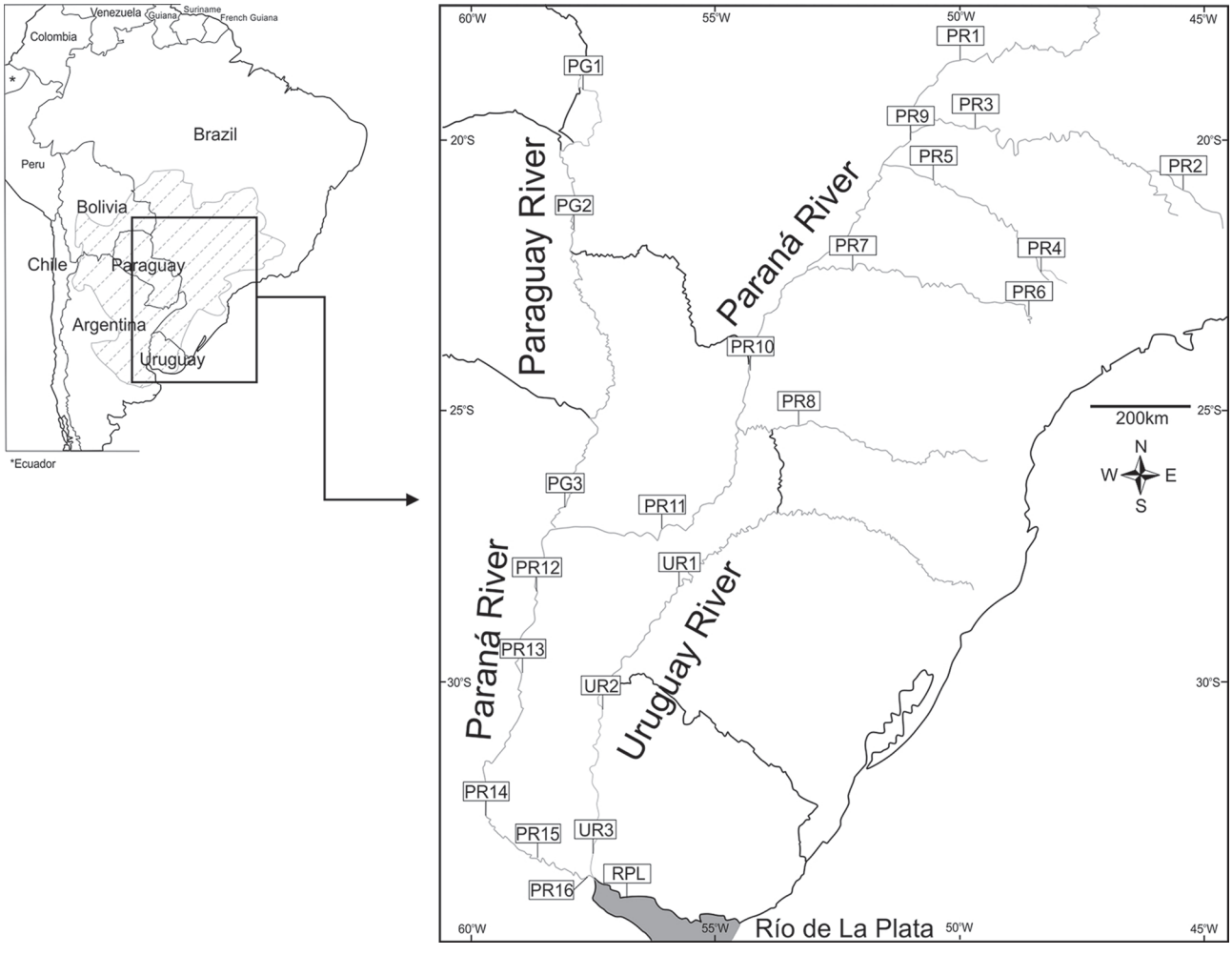

Figure 1. Sampling sites distribution in the Río de La Plata basin. PR1-10 = Upper Paraná sub-basin; PR11-13 = Middle Paraná sub-basin; PR14-16 = Low Paraná sub-basin; PG = Paraguay sub-basin; UR = Uruguay sub-basin; RPL = Río de La Plata.

Table 1. Study sites with codes, geographical position, sub-basin and level of knowledge concerning Cladocera from Río de La Plata basin.

Code	Latitude	Longitude	Sub-Basin	Knowledge
PR1	18°42'23.59"S	50°02'28.25"W	Upper Paraná	Well studied: Lansac-Tôha *et al.*(1997), Rocha and Güntzel (1999), Lima *et al.* (2003), Perbiche-Neves and Nogueira (2010), Rocha *et al.* (2011) etc.
PR2	20°58'39.97"S	45°31'33.05"W		
PR3	19°56'51.47"S	49°44'51.51"W		
PR4	20°11'42.10"S	50°59'04.82"W		
PR5	22°41'03.74"S	48°22'00.77"W		
PR6	20°55'34.36"S	50°34'33.65"W		
PR7	23°28'23.66"S	48°38'23.56'W		
PR8	22°38'02.87"S	52°09'39.88"W		
PR9	25°30'30.60"S	53°17'34.35"W		
PR10	24°29'57.44"S	54°17'51.79"W		
PR11	27°26'20.04"S	56°14'30.16"W	Middle Paraná	Well studied: Paggi (1972; 1973; 1975; 1995; 2004), José de Paggi and Paggi (2007), Paggi and José de Paggi (1974; 1990) etc.
PR12	28°29'33.95"S	59°02'24.47"W		
PR13	30°00'54.59"S	59°32'51.93"W		
PR14	32°44'07.16"S	60°43'10.12"W	Low Paraná	In need of studies: Paggi (2004) shows general information for all Argentina.
PR15	33°40'49.00"S	59°38'48.80"W		
PR16	33°56'49.45"S	58°27'07.47"W		
PG1	18°58'48.29"S	57°38'26.26"W	Paraguay	In need of studies: Frutos *et al.* (2006) have a large spatial range; Espíndola *et al.* (1996) have no Chydoridae identification; Neves *et al.* (2003) and Güntzel *et al.* (2010) show species lists
PG2	21°40'41.13"S	57°53'25.21"W		
PG3	26°52'10.45"S	58°19'54.12"W		
UR1	28°29'35.23"S	55°58'17.53"W	Uruguay	No work on Chydoridae was found
UR2	30°44'57.60"S	57°44'37.31"W		
UR3	33°49'40.48"S	58°25'41.52"W		
RPL	34°25'38.85"S	57°34'40.75"W	-	-

Van Damme, Sinev & Dumont, 2011 (Van Damme *et al.*, 2011) and, thus, is considered to belong to this genus. A third possible new species is listed [Tab. 2, *Chydorus* sp. (?)], but due to lack of material for detailed analysis, no further consideration could be performed.

The percentage contribution of new taxa and new records to the total richness observed per region is recorded in Fig. 4. Each of the two new species is widespread, occurring in 4 of the 5 sub-basins (Tab. 2), but neither occurs in the Río de La Plata itself, whose fauna was composed of *Chydorus pubescences* Sars, 1901, *Ephemeroporus acanthodes* Frey, 1982, *Euryalona orientalis* (Daday, 1898), *Karualona muelleri* (Richard, 1897), *Kurzia polyspina* Hudec, 2000, *Leydigia striata* Berabén, 1939, *Nicsmirnovius* sp. and *Picripleuroxus* cf. *similis* (Vávra, 1900).

Discussion

The main objective of this study was to perform an inventory of the Cladocera of the Río de La Plata basin and to analyze the contribution of new species to the fauna of different regions of the basin considered as having contrasting degrees of knowledge.

A large number of species was identified, many of them with taxonomic uncertainties.

This is in agreement with other recent research whose data suggest that *Alona broaensis* Smirnov and Matsumura-Tundisi, 1984 and *Alona dentifera* (Sars, 1901) are probable synonyms (Van Damme *et al.*, 2010), *Coronatella* cf. *poppei* (Richard, 1897) is a species complex being described (Sousa, unpublished data) and *Nicsmirnovius* sp. probably constitutes a new species in Brazil (Sousa *et al.*, 2012). It is important to note that *Nicsmirnovius* sp. does not correspond to the two known species from Americas: *Nicsmirnovius incredibilis* (Smirnov, 1984) and *Nicsmirnovius fitzpatricki* (Chien, 1970). Both *N. fitzpatricki* and *Nicsmirnovius* sp. are easily distinguished from *N. incredibilis* by the shape and armature of the postabdomen; while *N. incredibilis* has two distal angles and marginal clusters of setules, *N. fitzpatricki* and *Nicsmirnovius* sp. present a single distal angle and marginal denticles. *Nicsmirnovius* sp. differs from *N. fitzpatricki* in features of the trunk limbs (Sousa *et al.*, 2012).

Table 2. Species list with presence/absence of each species and total richness by sub-basin. UPR = Upper Paraná, MPR = Middle Paraná, LPR = Low Paraná, PG = Paraguay and UR = Uruguay. + presence; - absence. NR = New record for the Río de La Plata basin. Y = yes; N = no.

Species	UPR	MPR	LPR	PG	UR	NR
Aloninae						
Acroperus tupinamba Sinev and Elmoor-Loureiro, 2010	+	+	-	-	-	N
Alona sp.	+	-	+	+	+	Y
Alona broaensis Smirnov and Matsumura-Tundisi, 1984	+	-	-	-	-	N
Alona dentifera (Sars, 1901)	+	+	+	+	-	N
Alona glabra Sars, 1901	+	+	+	+	+	N
Alona guttata Sars, 1862	+	+	+	+	-	N
Alona iheringula Kotov and Sinev, 2004	+	-	-	+	-	N
Alona ossiani Sinev, 1998	+	+	-	+	-	N
Alona yara Sinev and Elmoor-Loureiro, 2010	+	+	-	+	-	N
Anthalona sp.	+	+	+	+	-	Y
Anthalona verrucosa (Sars, 1901)	+	+	+	-	-	N
Bergamina lineolata (Sars, 1901)	-	-	-	+	-	N
Camptocercus australis Sars, 1896	+	+	+	+	+	N
Coronatella cf. *poppei* (Richard, 1897)	-	-	-	+	-	N
Coronatella monacantha (Sars, 1901)	+	+	+	+	-	N
Coronatella cf. *rectangula* (Sars, 1861)	-	-	-	+	-	N
Euryalona brasiliensis Brehm and Thomsen, 1936	+	-	-	-	-	N
Euryalona orientalis (Daday, 1898)	+	+	+	+	+	N
Graptoleberis occidentalis Sars, 1901	+	+	+	+	-	N
Karualona muelleri (Richard, 1897)	+	+	+	+	-	N
Kurzia polyspina Hudec, 2000	+	+	+	+	-	N
Leberis davidi (Richard, 1895)	+	+	+	+	+	N
Leydigia striata Berabén, 1939	+	+	-	-	-	N
Leydigiopsis ornata Daday, 1905	+	+	+	+	-	N
Nicsmirnovius sp.	+	+	+	+	+	N
Notoalona sculpta (Sars, 1901)	+	+	+	+	-	N
Oxyurella ciliata Bergamin, 1939	-	+	-	+	-	N
Oxyurella longicaudis (Birge, 1910)	+	-	+	+	-	N
Parvalona parva (Daday, 1905)	-	-	-	+	-	N
Chydorinae						
Alonella clathratula Sars, 1896	-	+	-	-	-	N
Alonella dadayi Birge, 1910	+	+	+	+	+	N
Chydorus cf. *sphaericus* sens. lat.	+	-	-	-	-	N
Chydorus dentifer Daday, 1905	+	-	-	+	-	N
Chydorus eurynotus Sars, 1901	+	+	+	+	+	N
Chydorus nitidulus (Sars, 1901)	+	+	-	+	-	N
Chydorus parvireticulatus Frey, 1987	+	-	-	-	-	N
Chydorus pubescens Sars, 1901	+	+	+	+	+	N
Chydorus sp. (?)	+	-	-	+	-	Y
Dadaya macrops (Daday, 1898)	+	+	-	+	-	N
Disparalona cf. *hamata* (Birge, 1879)	-	-	-	-	+	N
Disparalona leptorhyncha Smirnov, 1996	+	-	-	-	-	N
Dunhevedia colombiensis Stingelin, 1913	-	+	+	+	-	Y
Dunhevedia crassa King, 1853	-	+	+	-	-	Y
Dunhevedia odontoplax Sars, 1901	+	+	+	+	-	N
Ephemeroporus barroisi-group (Richard, 1894)	-	-	-	-	+	N

Table 2. (Cont.)

Ephemeroporus cf. *acanthodes* Frey, 1982	-	-	-	+	-	Y
Ephemeroporus hybridus (Daday, 1905)	+	+	-	+	+	N
Ephemeroporus tridentatus (Bergamin, 1939)	+	+	-	+	+	N
Picripleuroxus cf. *similis* (Vávra, 1900)	-	+	+	+	-	N
Pseudochydorus globosus (Baird, 1850)	+	-	+	+	-	N
Richness	37	31	24	37	13	

Alona guttata Sars, 1862 is probably a species complex and populations outside its type locality (Østenjøvand, Norway) have dubious identity (Van Damme *et al.*, 2010). *Alonella lineoloata* Sars, 1901 has been recently redescribed and transferred to the new genus *Bergamina* Elmoor-Loureiro, Santos-Winiewsky & Rocha, 2013 (Elmoor-Loureiro *et al.*, 2013) as *Bergamina lineolata*. *Chydorus* sp. (?) is a probable new species with wide distribution in the continent since individuals with the same carapace ornamentation were found in Colombian Amazon (Elmoor-Loureiro, unpublished data), but due to lack of material, it is impossible at the moment to propose with certainty a new taxonomical status. *Chydorus eurynotus* Sars, 1901 observed here exhibits a fold of the ventral margin of carapace similar to *Chydorus ventricosus* Daday, 1898, which implies the need of reevaluation of these taxa for the basin. In the present study, one individual of the genus *Dunhevedia* King, 1853 presented the labral keel smooth, but it clearly was in the premolt stage and a single tooth was observed on the underlying new cuticle. Nowadays, some experts describe, when possible, not only the adult female, but also the male and juveniles (for instance: Sinev & Kotov, 2012) and the lack of information on the ontogeny is another constraint to the identification of species. Thus, it is possible that *Dunhevedia odontoplax* Sars, 1901 may have morphological variations during its ontogeny that resembles *Dunhevedia crassa* King, 1853. In addition, *D. crassa* and *Dunhevedia colombiensis* Stingelin, 1913 are new records for the basin. *Ephemeroporus* cf. *acanthodes* was described for North America, this being the first record of this species in the basin, with only one other record for South America (Lopez, 1993).

The proposal of two new species of Chydoridae in the Río de La Plata basin is based on morphological analyses: The *Alona*-like taxon showed ovoid body, with weak lateral compression, dorsal keel absent, arched, no posteroventral notch on valves; three main head pores, lateral pores minute; labral keel wedge-shaped to rounded and naked; postabdomen relatively short and robust, with deep pre-anal portion, unmerged marginal denticles; first limb presenting three setae; third limb with seven setae on the exopodite; fourth limb with six setae on the exopodite. In many aspects, this species resembles the members of the genus *Ovalona* Van Damme and Dumont, 2010. However, it also seems to be close to some American species of the *Alona pulchella* King, 1853-group, such as *Alona altiplana* Kotov, Sinev and Berrios, 2010 and *Alona anamariae* Sinev and Silva-Briano, 2012. This species is being described (Sousa, unpublished data). In the case of the *Anthalona* sp., all coarse morphological characteristics are similar to African *A. simplex* (Van Damme *et al.*, 2011). Recently, Sinev and Kotov (2012) described *Anthalona sanoamuang* Sinev and Kotov, 2012 from Indochina, other species with morphology resembling *A. simplex*. This finding may indicate a species group with circumtropical distribution. The investigation of fine characteristics, for instance all thoracic limbs, is under development, and indicates a new species (Sousa *et al.*, unpublished data). The investigation will allow us to obtain a better understanding of the relation among South American (this study), African (Van Damme *et al.*, 2011) and tropical Asia (Sinev and Kotov, 2012) taxa.

The results showed a relatively high contribution of the above cited taxa to the cladoceran assemblages of the Río de La Plata basin (Fig. 4). The presence of the two possible new species in the Paraná sub-basin was a surprising result, indicating that there is still some work to be done in the regions around research centers. Their lower proportional contribution when compared to other regions was due to a higher cladoceran richness in the basin, especially in the middle Paraná stretch.

The expected presence of new species in the Paraguay sub-basin was confirmed. The wetland-floodplain system in this area has some similarities with the fluvial system of the middle

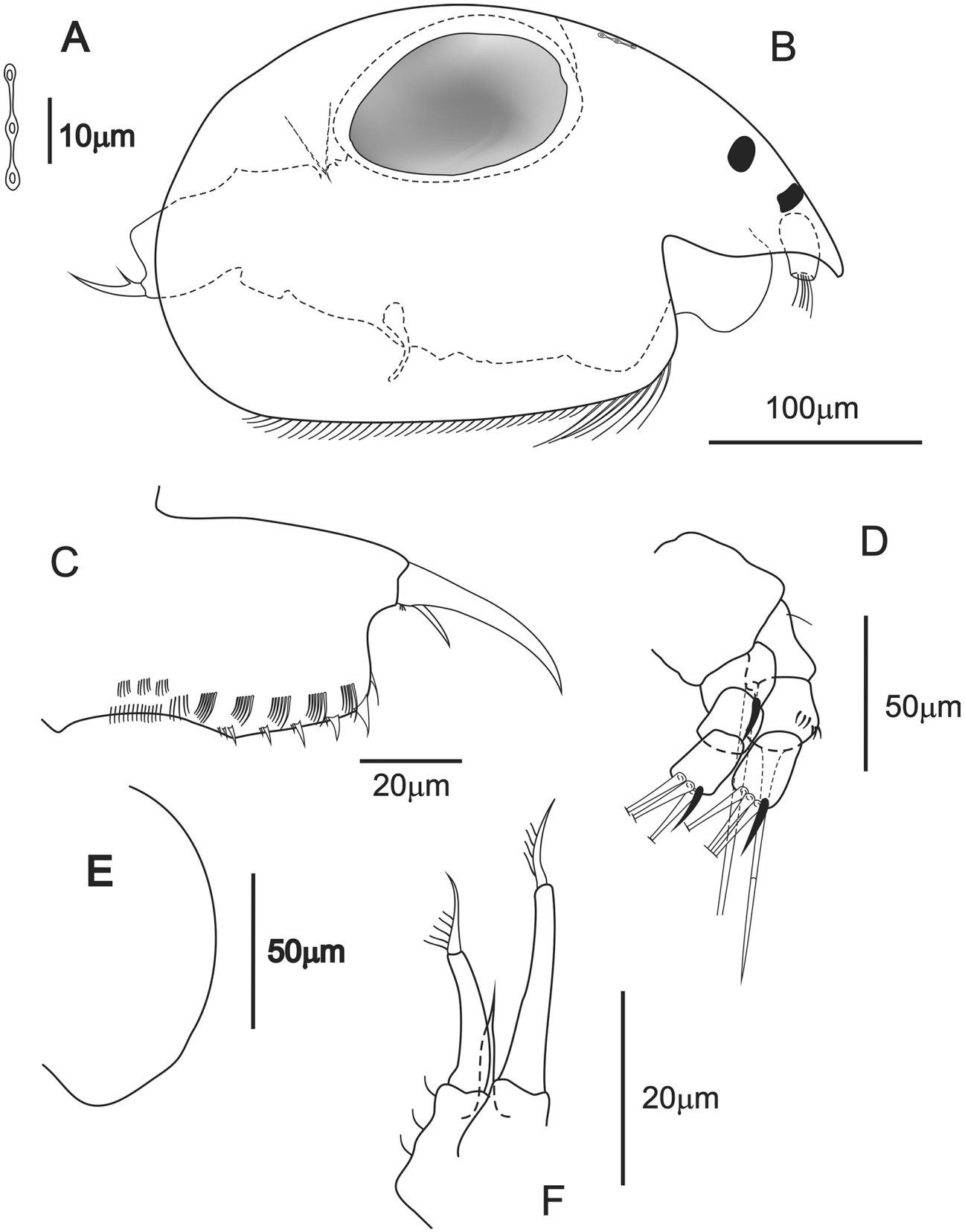

Figure 2. *Alona* sp. A: Main head pores; B: lateral view; C: postabdomen; D: antenna; E: labral kell; F: first limb, inner distal lobe (IDL).

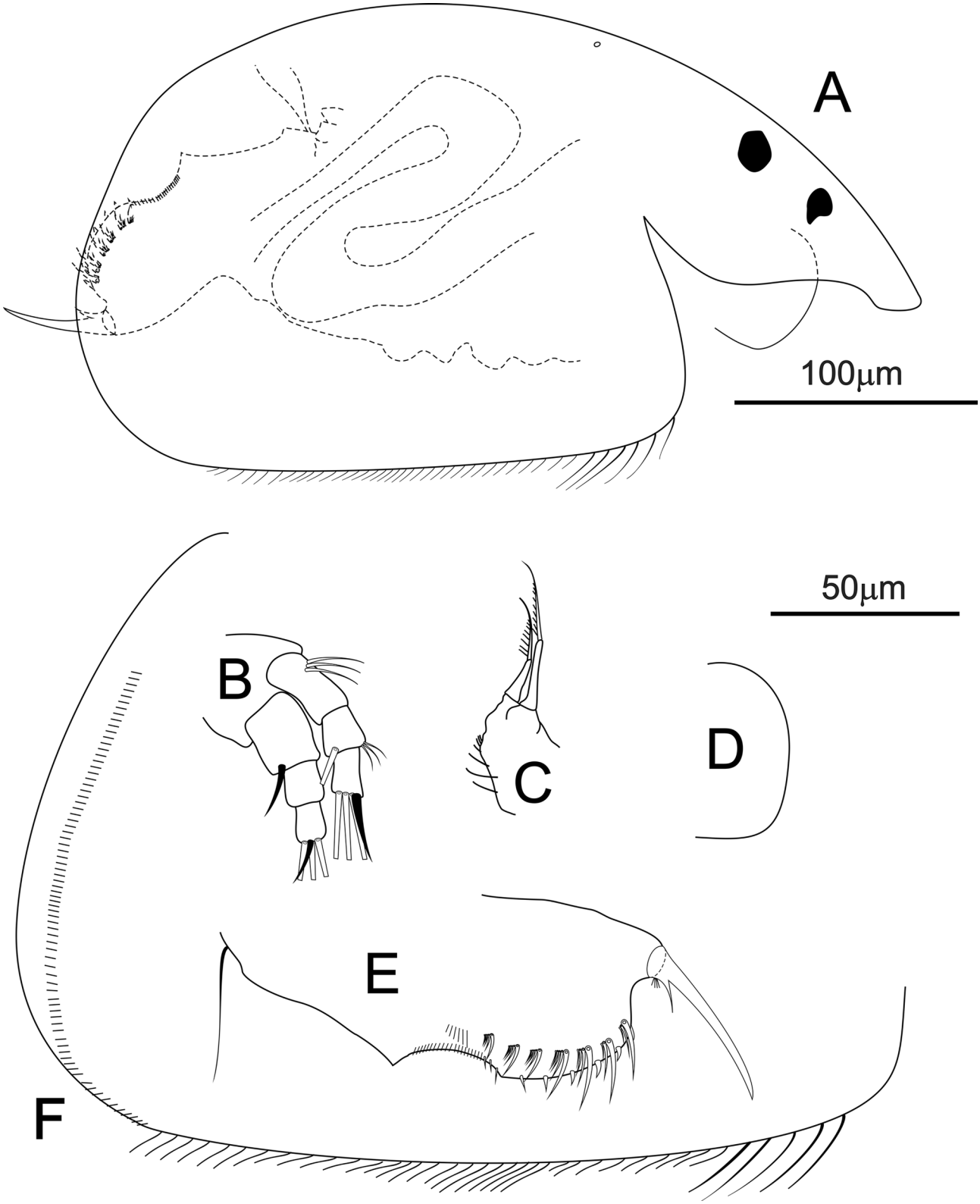

Figure 3. *Anthalona* sp. A: Lateral view; B: antenna; C: first limb, inner distal lobe (IDL); D: labral keel without denticle; E: postabdomen; F: ventral and posterior margins of carapace. Scale bars: A = 100 µm; B-F = 50 µm.

Paraná sub-basin, which exhibits a large richness. Therefore, the Paraguay sub-basin, and especially the Pantanal, a hotspot of biodiversity (Junk *et al.*, 2014), is in urgent need of investigation regarding their Cladocera fauna. This type of data help to support the decision making process on definition of conservation priorities and must be readily available with easy access through the scientific literature.

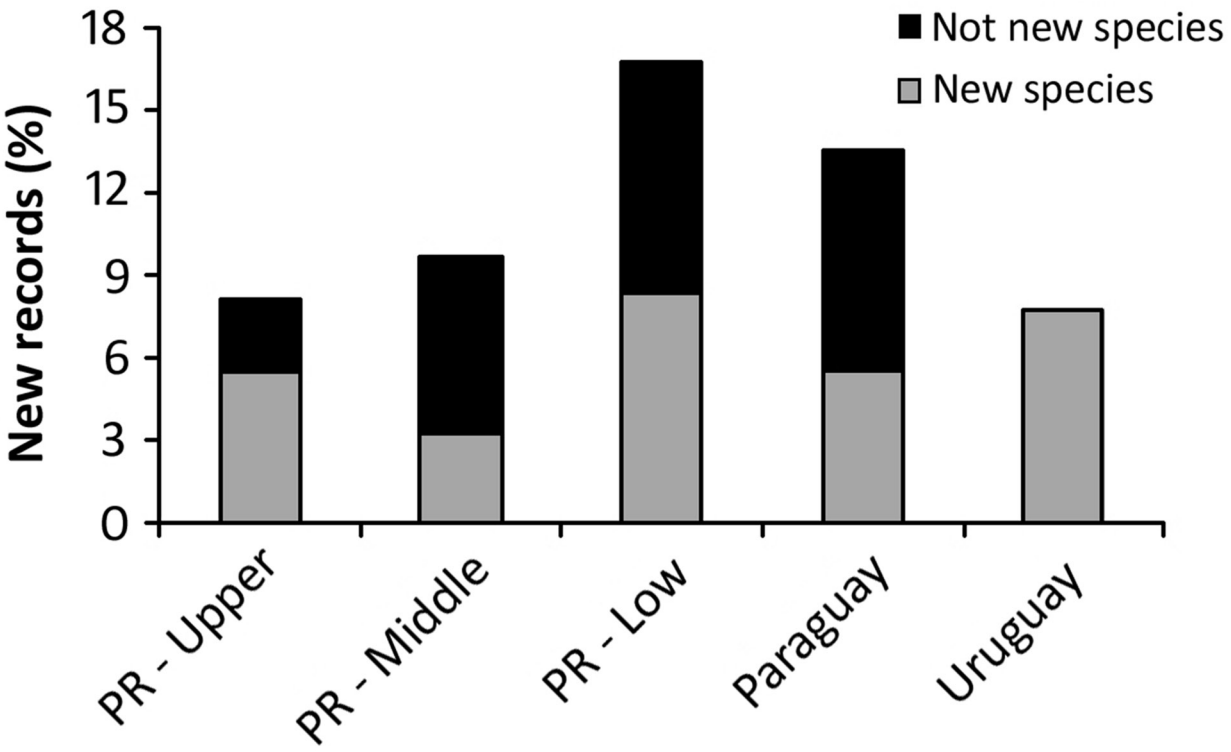

Figure 4. Relative contribution of the new records to the observed richness of the studied sub-basins. PR = Paraná.

Because it was not found any work on Chydoridae for the Uruguay sub-basin all the 13 species observed in this basin can be considered as new records for it. Nevertheless, 13 is a small number for such a large basin, being this probably an underestimate of the regional richness. For example, Serafim-Junior *et al.* (2006) brings a list of species from a neighbor basin containing only two overlapping species with ours (what would elevate the number to 18), what strengthens the possibility of an even higher number of actual resident species in the Uruguay sub-basin.

The sampling effort in this study could be questioned, producing as it did the typical dichotomy of wide spatial scale (geographical) and low temporal frequency. Nevertheless, the results highlight the importance of Cladocera, particularly Chydoridae, for invertebrate diversity in freshwater ecosystems of La Plata basin and how the taxonomy of this group in South America is a neglected research field when compared to other continents, especially North America and Europe where taxonomy developed earlier (Korovchinsky, 1997). In addition, it is worth noting the work of Sinev and Kotov (2012), which found new taxa in Thailand, though it is the most studied region of Indochina. Thus, the increase in taxa numbers due to higher taxonomy resolution is a worldwide phenomenon.

The resolution of identification, rather than the number of studies, seems to be a key factor determining the discovery of these taxa. This can explain the relative lack of influence of the "expected level of knowledge" on the finding of new species and highlight the need of expert taxonomists in South American Science. Chatterjee *et al.* (2013) discuss how taxonomic shallow analyses were detrimental to Cladocera research in India.

Nevertheless, the arrangement of the number of researches also necessarily influences the knowledge regarding Cladocera taxonomy and biogeography. For instance, Europe has a history of research that permits one to consider the continent well studied as cited above. On the other hand, other continents are recently being research targets as the cases of South America (e.g. Kotov *et al.*, 2010; Sinev and Elmoor-Loureiro, 2010; Elmoor-Loureiro *et al.*, 2013; Sousa *et al.*, 2013), Central America (e.g. Silva-Briano and Dumont, 2001; Elías-Gutiérrez and Valdez-Moreno, 2008; Elías-Gutiérrez *et al.*, 2001; 2006), Africa (Sinev 2009; Van Damme and Eggermont, 2011; Van Damme *et al.*, 2013) and Asia (e.g. Kotov *et al.*, 2013; Sinev and Korovchinsky, 2013), but these studies are not enough to represent the entire continents, with knowledge being restricted to few localities.

This results in fragmentary knowledge that hinders the possibility of detailed biogeographical analysis due to the lack of information regarding species range in those continents.

It is concluded that high resolution analyses applied to the Cladocera identification in the Río de La Plata basin, as well as in other regions, associated to extensive samplings are a crucial factor for the finding of new species and new records and for the advancement on taxonomy and biogeography knowledge. Knowledge concentration around few research centers, focused on particular localities results in limited taxonomical and biogeographical knowledge, being not advised to make considerations before introducing more data regarding the group in the literature.

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