"Taxonomy and ecology of ostracods (Crustacea, Ostracoda) from temporary waters of semi-arid areas in southern Africa"

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Freshwater ostracods (Ostracoda) are common microscopic crustaceans (usually 0.5 to 3.5 mm long) which differ from other groups by their fused body segmentation and the presence of a bivalve calcareous carapace covering the entire body. These invertebrates occur in almost all aquatic habitats, such as springs, streams, rivers, lakes, wetlands, human-constructed water impoundments of all sizes, permanent and temporary drying waters, slightly acidic to highly alkaline, as well as subterranean waters or even semi-terrestrial environments. Distribution of inland water ostracods has been proved to be controlled by several abiotic (e.g. water temperature, bottom sediment type, catchment geology, waterbody depth and size, or water chemistry) and biotic factors (e.g. vegetation). Ostracods are also one of the most important microfossils used in palaeolimnology, as their bivalved carapaces are calcified and often preserve well in Quaternary and older sediments, enabling reconstruction of past environmental changes (Smith et al. 2015).

The study area of the present study included semi-arid areas of Botswana and the North West Province of South Africa. Botswana is a landlocked country with a predominant warm, semi-arid climate and unpredictable rainfall, where frequent periods of drought, excessive grazing by animals and soil erosion further accelerate desertification of the country. The northeastern part of the country is slightly more humid than the southwest, where high evapotranspiration rates increase environmental dryness. The only permanent wetland areas are the Okavango and Chobe river systems in the north (Hughes & Hughes 1992). The North West Province of South Africa is mainly an agricultural region with relatively scarce and irregular rainfall and high temperatures, characterized by large monthly and daily fluctuations (Kabanda 2015). In the semi-arid areas most standing water bodies and watercourses are seasonal. These are mainly small, often saline pools periodically filled with rainwater, temporary rivers and artificial water reservoirs.

Seasonally drying water environments are characterized by the occurrence of dry phases of varying lengths, making them one of the most unpredictable and seemingly unsuitable ecosystems in the world. Despite being a significant part of the global landscape and of great importance for biodiversity conservation, they are highly threatened by global climate change and negative anthropogenic influences. One of the types of temporary waters are endorheic pans, which due to the lack of natural outflows, are mainly dependent on rainwater flowing from the direct catchment area. As a result of a high degree of evaporation,

they are often characterized by a relatively high salt concentrations in the substrate and water (Henri et al. 2014). Invertebrates living in temporary waters, exposed mainly to desiccation and changes in the chemical composition of water, display a range of specific life strategies and interesting adaptations to these harsh environmental conditions (Williams 2006).

The aims of this study were: a) faunistic survey with the description of species new to science, b) determination of the species composition and dominance structure of assemblages and c) examination how environmental factors influence the occurrence of ostracods in different types of temporary waters of semi-arid areas in southern Africa. The ostracods fauna of this region is still insufficiently known. There are only some preliminary faunistic reports and a few taxonomic studies available, while almost nothing is known about the environmental conditions determining occurrence of ostracods in this part of Africa.

The material for this study was collected from 39 sampling sites, representing predominantly different types of temporary waters in Botswana and the North West Province of South Africa. In total, 32,328 ostracod individuals belonging to 42 different species and 2,518 individuals of other macro- and meioinvertebrates from 28 families were collected, of which the vast majority (95%) were insects belonging to six orders (beetles, mayflies, butterflies, flies, true bugs and dragonflies).

Five species left in open nomenclature (*Hemicypris* sp., *Ilyocypris* sp., two different species of *Limnocythere* sp. and *Pseudocandona* sp.) are likely new to science and require detailed description, similar to what has been done for the parthenogenetically reproducing *Sarscypridopsis harundineti* Szwarc et al., 2021, *Potamocypris meissneri* Szwarc et al., 2021 and sexual *Pseudocypris* sp. described in the manuscript prepared for submission. The former species was collected from eight sites in floodplains on the outskirts of the Okavango Delta in northern Botswana. Individuals belonging to this species have been assigned to the typically southern African genus *Sarscypridopsis* based on the shape of the strongly reduced caudal ramus and the overlapping of the left valve by the right valve in the ventral part. Among other representatives of the genus, the new species is distinguished mainly by the characteristic shape of the carapace, which is more rounded and smaller. *Potamocypris meissneri* was described based on individuals found only in a small, temporary endorheic pan in the North West Province of South Africa. The specific features of this species are a conspicuously reticulate carapace, densely covered by prominent conuli carrying rimmed pores with long extending sensilla and by wide anterior and posterior flanges on the left valve. A detailed analysis of the diagnostic features of all species belonging to the genus *Potamocypris* known from southern Africa made it possible to present a key for their identification. The latter new

species found in the studied material, *Pseudocypris* sp. Szwarc and Namiotko (in preparation), was described from four temporary water bodies in the North West Province of South Africa. Individuals belonging to the genus *Pseudocypris* are quite easily recognizable based on their nearly flat ventral valve surface and the presence of characteristic lateral wing-like processes in the ventral half of the valves, present in almost all representatives. Due to relatively limited original descriptions of appendages (so-called soft parts), most species of this genus are distinguished based on carapace features and detailed morphology of male copulatory organs. *Pseudocypris* sp. is characterized by the presence of numerous and prominent spines on the valves, relatively broad lateral valve processes, as well as the species-specific shape of male copulatory organs and prehensile palps. All newly described species were illustrated in detail, both by taking pictures of the valves using a scanning electron microscope and by making drawings of all appendages and copulatory organs.

The total species richness of recent and fossil ostracods (preserved as calcareous valves in sediments of the Late Pleistocene to the Holocene) in Botswana was estimated based on our own material, which included 29 species from 19 sites, and the literature data from 15 papers concerning additional 12 sites. These data were used to compile a list of species with information on the geographical location and ecological characteristics of the sites. Most previous studies have focused on the northern part of the country, while our collections provided data from the south and east. In total, 54 ostracod species (45 recent and nine fossil) belonging to 22 genera of five families were recorded in Botswana, with almost 76% belonging to the family Cyprididae, which can be expected as species in this family produce resting eggs that allow them to survive the drying of water bodies and to passively disperse between isolated water systems. The most common species in Botswana were *Sarscypridopsis harundineti* and *Heterocypris oblonga* (Sars, 1924) found at nearly 29% of the sampled sites and *Potamocypris mastigophora* (Methuen, 1910), which was found at 21% of the sites. The taxonomic diversity of the Botswana ostracod fauna consists of both cosmopolitan species with wide geographic distributions, such as *Cypridopsis vidua* (O.F. Müller, 1776) and *Heterocypris incongruens* (Ramdohr, 1808), as well as three endemic species restricted to small areas: *Amphibolocypris arida* Jocqué & Martens, 2010, *Sarscypridopsis harundineti* and *Sclerocypris exserta makarikarensis* Martens, 1988. Nine of the species collected by us turned out to be a new for Botswana. Compared to the total species richness of Botswana (gamma diversity = 54 species), the species richness at individual sites (alpha diversity) was relatively low, ranging from 1 to 12 species, with an average of 3.3 species. Estimation of the total number of species based on the collected data using the Chao2 index revealed that the

observed total species richness of recent Ostracoda accounted for only 65% of the estimated species richness. The sampling sites were located within three Freshwater Ecoregions of the World (Okavango, Kalahari and Zambezian Lowveld). Analysis of similarity (ANOSIM) showed significant differences in the species composition of ostracod assemblages between the ecoregions. The occurrence of *S. harundineti* and *H. oblonga* largely contributed to the variation between the Okavango and Zambezian Lowveld ecoregions, as well as between the Okavango and Kalahari ecoregions. Similar results were obtained using the permutational analysis of multivariate dispersions (PERMDISP) to assess differences in beta diversity between the compared ecoregions.

In the North West Province of South Africa samples were collected from 20 sites, which were described based on environmental characteristics such as water body type, water chemistry, macrophyte coverage, substrate type, and the catchment use. In total, 28,088 individuals belonging to 16 species were collected of which 13 had never been recorded in this province before. The most numerous were representatives of the family Cyprididae (13 species, 81% of the total species richness) and the subfamily Cypridopsinae with 8 species constituting 50% of the total species number in this material, which corresponds to the species composition recorded in the ostracod fauna of Botswana and in the fauna of other better known provinces of South Africa (Martens et al. 1998; Namiotko et al. 2023). The most common species were *Heterocypris giesbrechti* (G.W. Müller, 1898), *Plesiocypridopsis newtoni* (Brady & Robertson, 1870) (13 sites, 65% of all sites) and *P. mastigophora* (11 sites, 55%), whereas seven species were found only at single sites. The number of species at individual sites (alpha diversity) ranged from 1 to 5, with an average of 3.6.

The natural water bodies sampled in the North West Province of South Africa represented three main types of endorheic waters (pans): grass, open and saline (de Klerk et al. 2016). Based on SIMPER analysis, it was found that ostracod assemblages in different types of waters differed more in dominance structure than in species composition. Saline endorheic pans were dominated by *H. giesbrechti* and *P. newtoni*, which together with *P. mastigophora* were also the most abundant in grass pans. The latter species had also the highest contribution in the assemblages of ostracods in open endorheic pans.

Among the functional feeding groups (FFGs) of macroinvertebrates (Merritt et al., 2017) accompanying ostracods, the most abundant were predators and collectors, accounting for 63% and 25% of the total abundance of macroinvertebrates, respectively. These two groups primarily explained the variation among the analyzed sites in the Principal Component Analysis (PCA), wherein the vectors of predator and collector abundances were directed

opposite to the gradient represented by the first ordination axis, which explained 86% of the total variation. Similar results were obtained in the PCA analysis based on taxon abundances, where the first axis explaining 35% of the total variation was positively correlated with a high abundance of predatory water boatman of the family Corixidae and negatively correlated with the abundance of Chironomidae larvae, representative of collectors.

The type of endoreic pans, water conductivity, and loadings for the sites along the gradient of the first axis of the FFG-based PCA ordination (PC1 FFG) were identified as statistically significant variables explaining the composition and dominance structure of the studied ostracod assemblages using Distance-based Linear Modeling (DistLM). In the best model according to the Akaike Information Criterion, only two variables were included: conductivity and PC1 FFG. The model was visualized using distance-based Redundancy Analysis (dbRDA), where the first axis was positively correlated with electrical conductivity of water and negatively correlated with PC1 FFG. This axis mainly distinguished saline endoreic water bodies with high water conductivity and a higher abundance of predatory macroinvertebrates from the other two types of endoreic water bodies, which were less clearly separated. Differences in species composition and dominance structure of ostracod assemblages among the different types of endoreic waters turned out to be statistically significant also in a multivariate permutation analysis of variance (PERMANOVA). Assemblages from saline water bodies, dominated by *H. giesbrechti* and *P. newtoni*, correlated with a high abundance of predatory invertebrates, while *P. mastigophora*, mainly inhabiting open water bodies, occurred more frequently with collectors. These results suggest that the structure of ostracod assemblages in southern African endoreic pans may be directly influenced by the specific predator selectivity and prey sensitivity, and that biotic factors may play a more important role in shaping ostracod assemblages than previously thought.

Summary

1. A total of 32,328 ostracods belonging to 42 different species were collected from 39 sites representing temporary water systems in the semi-arid areas of Botswana and North West Province of South Africa.
2. Three species of freshwater ostracods new to science were described from southern Africa.

3. All available data (own and historical) on the occurrence of ostracods in Botswana were collected and significant differences in species composition of assemblages between three freshwater ecoregions in this country were demonstrated.
4. It was found that ostracod assemblages occurring in different types of endorheic pans of the North West Province of South Africa differ more in dominance structure than in species composition, and the largest influence on the structure of ostracods have water chemistry (electrical conductivity) and biotic factors (abundance of macroinvertebrates accompanying ostracods, both predators and collectors).