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Taksonomia i ekologia małżoraczków (Crustacea, Ostracoda) wód okresowo wysychających półpustynnych obszarów południowej Afryki

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

Praca przedstawiona Radzie Dyscypliny Nauki biologiczne Uniwersytetu Gdańskiego celem uzyskania stopnia doktora w dziedzinie nauk ścisłych i przyrodniczych w dyscyplinie nauki biologiczne

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Słodkowodne małżoraczki (Ostracoda) to pospolite mikroskopijne skorupiaki (zwykle o długości od 0,5 do 3,5 mm), które odróżniają się od innych gromad zatartą segmentacją ciała oraz występowaniem dwuklapowego wapiennego karapaksu obejmującego całe ciało. Bezkręgowce te występują prawie we wszystkich siedliskach wodnych, takich jak źródła, strumienie, rzeki, jeziora, tereny podmokłe, sztucznie stworzone przez człowieka zbiorniki wodne każdej wielkości, wody trwałe i okresowo wysychające, o odczynie od lekko kwaśnego do silnie zasadowego, a także wody podziemne czy nawet środowiska ziemnowodne. Udowodniono, że występowanie poszczególnych gatunków i wielogatunkowych zgrupowań małżoraczków w wodach śródlądowych jest kontrolowane przez szereg czynników abiotycznych (np. temperatura wody, typ osadów dennych, geologia zlewni, głębokość i wielkość zbiorników wodnych czy chemizm wód) i biotycznych (np. typ roślinności). Małżoraczki są również jednymi z najważniejszych mikroskamieniałości wykorzystywanych w paleolimnologii, ponieważ ich zwapnione dwuklapowe pancerze często dobrze zachowują się w czwartorzędowych i starszych osadach, umożliwiając rekonstrukcję przeszłych zmian środowiskowych (Smith i in. 2015). Teren niniejszych badań obejmował półpustynne obszary Botswany oraz Prowincji Północno-Zachodniej Republiki Południowej Afryki. Botswana to kraj śródlądowy z dominującym ciepłym, półpustynnym klimatem i nieprzewidywalnymi opadami deszczu, gdzie częste okresy suszy oraz nadmierny wypas zwierząt i erozja gleby dodatkowo przyspieszają pustynnienie kraju. Północny wschód kraju jest nieco bardziej wilgotny niż południowy zachód, gdzie wysokie wskaźniki ewapotranspiracji zwiększają suchość środowiska. Jedynymi stałymi terenami podmokłymi są systemy rzek Okawango i Chobe na północy (Hughes i Hughes 1992). Prowincja Północno-Zachodnia RPA to w głównej mierze rolniczy obszar o dość skąpych i nieregularnych opadach oraz wysokich temperaturach o dużych miesięcznych i dziennych wahaniach (Kabanda 2015). Na terenach półpustynnych większość zbiorników i cieków wodnych ma charakter okresowy. Są to głównie małe, zasolone zbiorniki czasowo wypełniane wodą deszczową, tymczasowe rzeki oraz sztucznie stworzone rezerwuary wody.

Środowiska wód okresowo wysychających charakteryzują się występowaniem mniej lub bardziej regularnie powtarzającej się fazy suchej o różnej długości, co czyni je jednymi z najbardziej nieprzewidywalnych i pozornie nieodpowiednich do życia ekosystemów na świecie. Pomimo tego, że takie środowiska wodne stanowią znaczną część światowego krajobrazu i mają ogromne znaczenie dla zachowania różnorodności biologicznej, są one w dużym stopniu zagrożone globalnymi zmianami klimatycznymi i negatywnymi wpływami antropogenicznymi. Jednym z typów wód okresowych są zbiorniki endoreiczne (bezodpływowe), które ze względu na brak naturalnego odpływu są w głównej mierze uzależnione od wody opadowej spływającej z bezpośredniej zlewni. W wyniku wysokiego stopnia parowania charakteryzują się one często relatywnie dużym stężeniem soli w podłożu i w wodzie (Henri i in. 2014). Bezkręgowce żyjące w wodach okresowych, narażone głównie na wysychanie oraz zmiany składu chemicznego wody, wykazują szereg specyficznych strategii życiowych i ciekawych przystosowań do tych trudnych warunków środowiskowych (Williams 2006).

Celem niniejszych badań było: a) rozpoznanie faunistyczne z opisem gatunków nowych dla nauki, b) określenie składu gatunkowego i struktury dominacyjnej zgrupowań oraz c) zbadanie wpływu czynników środowiskowych na występowanie małżoraczków (Ostracoda) w różnych typach wód okresowo wysychających półpustynnych obszarów południowej Afryki. Fauna Ostracoda tego regionu w dalszym ciągu jest niewystarczająco dobrze poznana. Dysponujemy wyłącznie przyczynkowymi doniesieniami faunistycznymi oraz nielicznymi pracami taksonomicznymi, natomiast prawie nic nie wiadomo o uwarunkowaniach środowiskowych występowania małżoraczków w tej części Afryki.

Materiał do niniejszych badań został pobrany z 39 stanowisk reprezentujących niemal wyłącznie różne środowiska wód okresowo wysychających w Botswanie oraz Prowincji Północno-Zachodniej RPA. Łącznie zebrano 32 328 osobników małżoraczków należących do 42 różnych gatunków oraz 2 518 osobników innych makro- i meiobezkręgowców z 28 rodzin, z czego zdecydowaną większość (95%) stanowiły owady należące do sześciu rzędów (chrząszcze, jętki, motyle, muchówki, pluskwiaki i ważki).

W obrębie całego oznaczonego materiału pięć gatunków pozostawionych w otwartej nomenklaturze (Hemicypris sp., Ilyocypris sp., dwa różne gatunki Limnocythere sp. i Pseudocandona sp.) to najprawdopodobniej gatunki nowe dla nauki i wymagają szczegółowego opisu, tak jak zostało to wykonane w przypadku partenogenetycznie rozmnażających się Sarscypridopsis harundineti Szwarc i in., 2021, Potamocypris meissneri Szwarc i in., 2021 oraz opisanego w manuskrypcie przygotowanym do druku Pseudocypris sp. Pierwszy z nich został zebrany na ośmiu stanowiskach z obszarów zalewowych na obrzeżach delty Okawango w północnej Botswanie. Osobniki należące do tego gatunku zostały oznaczone do typowo południowoafrykańskiego rodzaju Sarscypridopsis na podstawie kształtu silnie zredukowanych widełek ogonowych oraz obejmowania lewej skorupki przez prawą w części brzusznej. Wśród innych przedstawicieli rodzaju nowy gatunek wyróżnia się głównie charakterystycznym kształtem karapaksu, który jest bardziej zaokrąglony i mniejszymi rozmiarami. Potamocypris meissneri został opisany na podstawie osobników znalezionych tylko w jednym niewielkim i okresowo wysychającym zbiorniku endoreicznym w Prowincji Północno-Zachodniej RPA. Swoistymi cechami tego gatunku są okazała ornamentacja skorupek, które pokryte są długimi szczecinami sensorycznymi wystającymi ze stożkowatych porów oraz obecność szerokiego kołnierza na przedniej i tylnej części lewej skorupki. Szczegółowa analiza cech diagnostycznych wszystkich gatunków należących do rodzaju Potamocypris znanych z południowej Afryki umożliwiła sporządzenie klucza do ich oznaczania. Kolejnym nowym dla nauki gatunkiem znalezionym w badanym materiale jest Pseudocypris sp. Szwarc i Namiotko (w przygotowaniu) opisany z czterech zbiorników okresowo wysychających w Prowincji Północno-Zachodniej RPA. Osobniki należące do rodzaju Pseudocypris są dość łatwo rozpoznawalne na podstawie niemal płaskiej brzusznej części skorupek oraz charakterystycznych bocznych skrzydlatych wyrostków w brzusznej połowie skorupek występujących u prawie wszystkich przedstawicieli. Ze względu na dosyć skąpe oryginalne opisy odnóży (tzw. części miękkich), większość gatunków tego rodzaju jest rozróżniana na podstawie cech karapaksu oraz szczegółów morfologii narządów kopulacyjnych samców. *Pseudocypris* sp. charakteryzuje się występowaniem licznych i wydatnych kolców na skorupce oraz relatywnie szerokimi bocznymi wyrostkami skorupek, a ponadto ma gatunkowo specyficzne kształty narządów kopulacyjnych oraz narządów chwytnych samców. Wszystkie nowo opisane gatunki zostały w szczegółowy sposób zilustrowane, zarówno poprzez zrobienie zdjęć skorupek przy użyciu skaningowego mikroskopu elektronowego, jak i wykonanie rysunków wszystkich odnóży i narządów płciowych.

Całkowite bogactwo gatunkowe współczesnych oraz fosylnych (występujących jedynie w postaci wapiennych skorupek zachowanych w osadach od późnego plejstocenu do holocenu) małżoraczków Botswany zostało oszacowane na podstawie własnego materiału obejmującego 29 gatunków z 19 stanowisk oraz danych literaturowych zaczerpniętych z 15 publikacji dotyczących kolejnych 12 stanowisk. Dane te posłużyły to opracowania listy gatunków z informacjami dotyczącymi położenia geograficznego i charakterystyki ekologicznej stanowisk. Większość dotychczasowych badań skupiała się na północnej części kraju, podczas gdy nasze zbiory dostarczyły danych z południa i wschodu. Łacznie w Botswanie stwierdzono występowanie 54 gatunków Ostracoda (45 współczesnych i dziewięć fosylnych) należących do 22 rodzajów z pięciu rodzin, z czego prawie 76% to przedstawiciele rodziny Cyprididae, czego można się było spodziewać, ponieważ gatunki należące do tej rodziny produkują jaja przetrwalne, które pozwalają im przetrwać wyschnięcie zbiornika oraz pasywnie pokonywać przestrzenie między izolowanymi od siebie systemami wodnymi. Najbardziej pospolitymi gatunkami w wodach Botswany były S. harundineti występujący na niemal 29% zbadanych stanowisk oraz Heterocypris oblonga (Sars, 1924) i Potamocypris mastigophora (Methuen, 1910), które zostały znalezione na 21% stanowisk. Na zróżnicowanie taksonomiczne botswańskiej fauny składają się zarówno kosmopolityczne gatunki o szerokim występowaniu geograficznym, np. Cypridopsis vidua (O.F. Müller, 1776) i Heterocypris incongruens (Ramdohr, 1808), jak i trzy endemiczne, ograniczone do niewielkich obszarów -Amphibolocypris arida Jocqué & Martens, 2010, S. harundineti i Sclerocypris exserta makarikarensis Martens, 1988. Dziewięć spośród zebranych przez nas gatunków okazało się nowymi dla Botswany. W porównaniu z całkowitym bogactwem gatunkowym Botswany (różnorodność gamma = 54 gatunki) liczebność gatunków na poszczególnych stanowiskach (różnorodność alfa) była relatywnie niska i wahała się od 1 do 12 gatunków, przy średniej równej 3,3. Estymacja całkowitej liczby gatunków na podstawie zebranych danych przy użyciu wskaźnika Chao2 ujawniła, że całkowite obserwowane bogactwo gatunkowe współczesnych Ostracoda stanowiło tylko 65% szacowanego bogactwa gatunkowego. Stanowiska poboru prób znajdowały się w obrębie trzech słodkowodnych ekoregionów, ang. *Freshwater Ecoregions of the World* (Okavango, Kalahari i Zambezian Lowveld). Na podstawie analizy podobieństw ANOSIM (ang. *Analysis of similarity*) wykazano istotne różnice w składzie gatunkowym zgrupowań Ostracoda pomiędzy ekoregionami. Występowanie gatunków *S. harundineti* i *H. oblonga* w głównej mierze wpłynęło na zróżnicowanie pomiędzy ekoregionami Okavango i Zambezian Lowveld oraz pomiędzy Okavango i Kalahari. Podobne wyniki uzyskano po zastosowaniu permutacyjnej analizy dyspersji wielowymiarowych PERMDISP (ang. *Permutational analysis of multivariate dispersions*) do oceny różnic w różnorodności beta pomiędzy porównywanymi ekoregionami.

W Prowincji Północno-Zachodniej RPA z 20 stanowisk opisanych pod względem środowiskowym (typ zbiornika, chemizm wód, pokrycie makrofitami, rodzaj podłoża oraz wykorzystanie zlewni) zebrano 28 088 osobników należących do 16 gatunków, z czego 13 nigdy wcześniej nie było notowanych w tej prowincji. Najbardziej liczni byli przedstawiciele rodziny Cyprididae (13 gatunków, 81% całkowitego bogactwa gatunkowego) oraz podrodziny Cypridopsinae z 8 gatunkami stanowiącymi 50% całego materiału, co odpowiada składowi gatunkowemu odnotowanemu w faunie małżoraczków Botswany oraz w faunie innych lepiej poznanych prowincji RPA (Martens i in. 1998; Namiotko i in. 2023). Najczęściej były Heterocypris giesbrechti występującymi gatunkami (G.W. Müller, 1898) i Plesiocypridopsis newtoni (Brady & Robertson, 1870) (13 stanowisk, 65% wszystkich) oraz P. mastigophora (11 stanowisk, 55%), natomiast aż siedem gatunków zostało znalezionych tylko na pojedynczych stanowiskach. Różnorodność alfa podczas obu zbiorów wahała się od 1 do 5 gatunków na stanowisku, ze średnią równą 3,6.

Naturalne zbiorniki wodne, z których zebrano próby w Prowincji Północno-Zachodniej RPA reprezentowały trzy główne typy wód endoreicznych (ang. *pans*): trawiaste, otwarte i zasolone (de Klerk i in. 2016). Na podstawie analizy SIMPER (ang. *Similarity Percentage*) stwierdzono, że zgrupowania małżoraczków występujące w różnych typach wód w większym stopniu różnią się strukturą dominacji niż składem gatunkowym. Zasolone zbiorniki endoreiczne zostały zdominowane przez *H. giesbrechti* oraz *P. newtoni*, które wraz z *P. mastigophora* były najliczniejsze również w zbiornikach trawiastych. Ten ostatni gatunek miał najwyższy udział także w zgrupowaniach małżoraczków otwartych zbiorników endoreicznych.

Wśród towarzyszących małżoraczkom funkcjonalnych grup troficznych (ang. *functional feeding groups* FFG) makrobezkręgowców (Merritt i in. 2017) najliczniejsi okazali się drapieżcy oraz zbieracze, stanowiące odpowiednio 63% i 25% całkowitej liczebności

makrobezkręgowców. Te dwie grupy w największym stopniu tłumaczyły zróżnicowanie analizowanych stanowisk w analizie głównych składowych PCA (ang. *Principal component analysis*), przy czym wektory udziału drapieżców i zbieraczy były skierowane przeciwnie w stosunku do gradientu reprezentowanego przez pierwszą oś ordynacyjną tłumaczącą 86% całkowitej zmienności. Podobne wyniki uzyskano w analizie PCA wykonanej na podstawie abundancji taksonów, w której pierwsza oś tłumacząca 35% zmienności była pozytywnie skorelowana z wysokim udziałem drapieżnych pluskwiaków z rodziny wioślakowatych (Corixidae) i negatywnie z udziałem larw ochotkowatych (Chironomidae), przedstawicielami zbieraczy.

Typ zbiornika endoreicznego, konduktywność wody oraz wskaźniki dla stanowisk w gradiencie pierwszej osi przestrzeni ordynacyjnej PCA wykonanej na podstawie FFG (PC1 FFG) zostały zidentyfikowane w modelowaniu liniowym opartym na miarach dystansowych DistLM (ang. Distance based Linear Modeling) jako statystycznie istotne zmienne wyjaśniające skład i strukturę dominacyjną badanych zgrupowań małżoraczków, przy czym w najlepszym modelu wg Akaike Information Criterion znalazły się tylko dwie zmienne: konduktywność i PC1 FFG. Wizualizację modelu przeprowadzono za pomocą analizy redundancji opartej na miarach dystansowych dbRDA (ang. distance based Redundancy Analysis), w której pierwsza oś była dodatnio skorelowana z przewodnością elektryczną wody a ujemnie z osią PC1 FFG i w głównej mierze wyraźnie oddzielała zasolone zbiorniki endoreiczne o wysokich wartościach konduktywności i większym udziale drapieżnych makrobezkręgowców od pozostałych dwóch typów zbiorników endoreicznych, rozdzielonych w mniejszym stopniu. Różnice w składzie gatunkowym i strukturze dominacyjnej zgrupowań małżoraczków poszczególnych typów wód endoreicznych okazały się istotne statystycznie w wieloczynnikowej permutacyjnej analizie wariancji PERMANOVA. Zgrupowania z zasolonych zbiorników, zdominowane przez H. giesbrechti i P. newtoni, korelują z wysokim udziałem drapieżnych bezkręgowców, natomiast P. mastigophora zasiedlający głównie zbiorniki otwarte występuje w większej mierze wraz ze zbieraczami. Wyniki te mogą wskazywać, iż na strukturę zgrupowań małżoraczków wód endoreicznych południowej Afryki może mieć bezpośredni wpływ specyficzna selektywność drapieżnika i wrażliwość ofiary, a także, że czynniki biotyczne mogą być ważniejsze w kształtowaniu zgrupowań Ostracoda, niż wcześniej sądzono.

Podsumowanie

- Z 39 stanowisk reprezentujących okresowo wysychające systemy wodne w półpustynnych obszarach Botswany i Prowincji Północno-Zachodniej RPA zebrano łącznie 32 328 osobników małżoraczków należących do 42 różnych gatunków.
- 2. Opisano trzy nowe dla nauki gatunki słodkowodnych małżoraczków z południowej Afryki.
- Zebrano wszystkie dostępne dane (własne i historyczne) dotyczące występowania Ostracoda w Botswanie i wykazano istotne różnice w składzie gatunkowym zgrupowań pomiędzy trzema słodkowodnymi ekoregionami w tym kraju.
- 4. Stwierdzono, że zgrupowania małżoraczków występujące w różnych typach wód endoreicznych Prowincji Północno-Zachodniej RPA w większym stopniu różnią się strukturą dominacji niż składem gatunkowym, a największy wpływ na strukturę zgrupowań mają chemizm wód (konduktywność elektryczna) oraz czynniki biotyczne (udziały towarzyszących małżoraczkom makrobezkręgowców, zarówno drapieżnych, jak i zbieraczy).

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Summary of the doctoral dissertation "Taxonomy and ecology of ostracods (Crustacea, Ostracoda) from temporary waters of semi-arid areas in southern Africa" Agata Szwarc, M.Sc

Scientific articles included in the doctoral dissertation:

- Szwarc A., Martens K., Namiotko T. 2021. Two new Cypridopsinae Kaufmann, 1900 (Crustacea, Ostracoda) from southern Africa. *ZooKeys* 1076: 83-107. https://doi.org/10.3897/zookeys.1076.76123
- Szwarc A., Namiotko T. 2022. Biodiversity of non-marine Ostracoda (Crustacea) of Botswana: an annotated checklist with notes on distribution. *Water* 14(9), 1441. https://doi.org/10.3390/w14091441
- Szwarc A., Martens K., Meissner W., Namiotko T. 2023. Evidence for conductivity- and macroinvertebrate-driven segregation of ostracod assemblages in endorheic depression wetlands in North West Province of South Africa. *Diversity* 15(5):614. https://doi.org/10.3390/d15050614
- Szwarc A., Namiotko T. 2023. On a new species of *Pseudocypris* Daday, 1910 (Crustacea, Ostracoda) from South Africa – manuscript

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

- 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).

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RESEARCH ARTICLE



Two new Cypridopsinae Kaufmann, 1900 (Crustacea, Ostracoda) from southern Africa

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Abstract

Two new Cypridopsinae ostracods, *Potamocypris meissneri* **sp. nov.** and *Sarscypridopsis harundineti* **sp. nov.** are described. Both were found only as asexual (all-female) populations in temporary waters of southern Africa. *Potamocypris meissneri* was collected from a small pan in the North-West Province of South Africa. It is approximately 0.5 mm long and belongs to the species group with long swimming setae on the second antennae. However, the species has a somewhat isolated position in the genus owing to the conspicuously reticulated carapace, which is furthermore densely covered by prominent conuli with normal pores carrying long sensilla, as well as to the wide anterior and posterior flanges on the left valve. To allow identification of the new species in relation to its closest congeners, a key to the species of the genus *Potamocypris* Brady, 1870 from southern Africa is provided. The genus *Sarscypridopsis* McKenzie, 1977 mostly has an Afrotropical distribution with only few species occurring in other regions. *Sarscypridopsis harundineti* was collected from floodplains of the outskirts of the Okavango Delta in Botswana. It is approximately 0.4 mm long and can be distinguished from congeners mainly by the smaller and more oval-shaped valves. We conclude that southern African Cypridopsinae urgently need integrated taxonomic revision, by means of both morphological characters and DNA-sequence data.

Keywords

Afrotropical, Cyprididae, microcrustaceans, morphology, taxonomy, temporary waters

Introduction

Ostracods, small bivalved crustaceans, have an impressive taxonomic diversity and functional specialisation of their appendages, which are used for locomotion, feeding, and reproduction (Meisch 2000; Smith et al. 2015). They commonly occur in both marine and non-marine habitats, from the oceans and estuaries, over deep lakes to small temporary pools, phytotelmata or troughs, as well as subterranean waters and even semi-terrestrial environments (Mesquita-Joanes et al. 2012; Smith et al. 2015). Ostracoda differ from (most) other crustaceans by a combination of two main features: firstly, by their body oligomerisation with no true body segmentation and secondly, by the strong development of the carapace consisting of two calcified valves hinged along the dorsal margin, and with central adductor muscles attached to the inner part of the valves, crossing the body from one valve to the other and creating characteristic internal muscle scar patterns on them.

Ostracoda are the extant arthropod group with the most abundant fossil record. Although much less diversified than marine lineages, freshwater ecosystems are home to ~ 2300 Recent (living) species and 270 genera (Meisch et al. 2019). Sixteen families have representatives in non-marine habitats, the most diverse family being the Cyprididae Baird, 1845. It includes 24 subfamilies of which the subfamily Cypridopsinae Kaufmann, 1900 is the richest (Meisch et al. 2019) with 22 genera (Savatenalinton 2018; Meisch et al. 2019; Savatenalinton 2020; Almeida et al. 2021). Cypridopsinae are mostly small animals (< 1.0 mm), characterised by a strong reduction of the caudal ramus, which is usually flagellum-like (or even missing) in females, and integrated in the hemipenes in males (Martens and Meisch 1985).

Potamocypris Brady, 1870, is after *Cypridopsis* Brady, 1867, the second most abundant genus within the subfamily (Meisch et al. 2019). The 46 species it includes are characterised by 1) asymmetrical valves, with the right valve overlapping the left one dorsally and ventrally, 2) a distinguishing spatula-like shape of the distal palp segment of the maxillula, and 3) a distally tapering caudal ramus. The genus has nearly cosmopolitan distribution, but only 10 species have so far been recorded from the Afrotropical region, and only five from southern Africa (Martens 2001; Meisch et al. 2019).

Sarscypridopsis McKenzie, 1977 is mostly distributed in South Africa, with 13 out of the 17 known species described from this country (Sars 1924a, 1924b). Similar to *Potamocypris*, in *Sarscypridopsis* the right valve overlaps the left valve anteriorly, ventrally and posteriorly, but the terminal segment of the maxillular palp is cylindrical and the base of the caudal ramus is triangular (McKenzie 1977).

Here, we describe one species each belonging to *Potamocypris* and to *Sarscypridopsis*. The present paper also constitutes a contribution to the knowledge of the poorly known freshwater ostracod fauna of southern Africa and presents the first comprehensive description of a species of the genus *Sarscypridopsis* with full illustration of valves and appendages.

Materials and methods

Samples were collected from temporary waters in South Africa and Botswana (Fig. 1) using a hand-net (mesh size of 120 µm) to sweep the bottom surface and among vegetation at the depth of < 50 cm. Sediment samples were rinsed in the field, placed in plastic jars and preserved in 96% ethanol. Physical and chemical properties of the pond water (temperature, pH and electrical conductivity) were measured in situ using a hand-held multi-parameter probe WTW Multi 350i. In the laboratory, samples were thoroughly rewashed with tap water through a 120 µm-mesh sieve, placed in plastic jars and preserved in fresh 96% ethanol. Specimens were sorted, counted, dissected, and mounted using a binocular and light transmission microscope according to Namiotko et al. (2011). Soft parts of dissected ostracods were mounted in glycerin or Hydro-Matrix mounting medium, whereas valves were stored dry on micropalaeontological slides. Species identification was performed using the keys published in McKenzie (1977), Meisch (2000), and Martens (2001), and taxonomical descriptions in Meisch et al. (2019). Drawings of soft parts were made with a camera lucida on a transmission light microscope Nikon Eclipse 50i (Univ. Gdansk, Poland). Carapaces and valves were gold-coated and observed under the scanning electron microscope, Fei Qanta 200 ESEM, at the Royal Belgian Institute of Natural Sciences, Brussels, Belgium as well as under a transmission light microscope Nikon Eclipse 50i (Univ. Gdansk, Poland).

The type specimens are deposited in the Collection of the Royal Belgian Institute of Natural Sciences, Brussels (**RBINS**, general I.D. 3439) and in the Ostracod Collection of the Laboratory of Biosystematics and Ecology of Aquatic Invertebrates, Department of Evolutionary Genetics and Biosystematics, University of Gdansk (**OC-UG**).

Chaetotaxy of the limbs follows the model proposed by Broodbakker and Danielopol (1982), revised for the second antenna by Martens (1987). Names for the limbs were used according to Meisch (2000) except for caudal ramus, which follows Meisch (2007).

Abbreviations used in text and figures

Limbs:

Α	anterior
a, a'	two setae on Pr of T1
A1	first antenna (antennule)
A2	second antenna
alfa (a)	special seta on the 1 st podomere of Md palp
beta (β)	special seta on the 2 nd podomere of Md palp

CR	caudal ramus
D	distal
d1, d2, dp	setae on Pr of T2 or T3
E	endopod
e	setae on EI of T2 and T3
EI-EIV	1 st to 4 th podomeres of E
Ex	exterior
Exo	exopod
f	setae on EII of T2 and T3
g	setae on EIII of T2 and T3
gamma (γ)	special seta on 3 rd podomere of Md palp
GM (Gm)	major (minor) claw on EIV of A2
G1–3	anterior and internal claws (or setae) on EIII of A2
h1–3	setae (or claws) on EIV of T2 and T3
In	interior
1	large (relative length of setae or claws)
m	medium (relative length of setae or claws)
Mastic	masticatory process on Pr of T1
Md	mandibula
Mx1	maxillula
Р	posterior
pl	plumed
Pr	protopod
s	small (relative length of setae or claws)
S1–2	plumed setae on 1 st podomere of Md palp
ser	serrated
T1	first thoracopod (maxilliped)
T2	second thoracopod (walking leg)
T3	third thoracopod (cleaning leg)
t1-4	internal setae on EII of A2
Y	aesthetasc on EI of A2
y1–3	aesthetascs on EII, EIII and EIV of A2 respectively
ya	aesthetasc on the terminal podomere of A1
z1–3	external setae (or claws) on EIII of A2

Valves and carapace:

- **Cp** carapace
- H valve height
- L valve length
- LV left valve
- **RV** right valve



Figure 1. Localities of *Potamocypris meissneri* sp. nov. (purple star SA-9) in the North-West Province of South Africa and *Sarscypridopsis harundineti* sp. nov. (red dots SA-96 to SA-103) in the outskirts south of the Okavango Delta in Botswana. The type locality of *Sarscypridopsis harundineti* sp. nov. (SA-103) is marked with a dark red dot.

Taxonomy

Class Ostracoda Latreille, 1802 Subclass Podocopa Sars, 1866 Order Podocopida Sars, 1866 Suborder Cypidocopina Baird, 1845 Superfamily Cypridoidea Baird, 1845 Family Cyprididae Baird, 1845 Subfamily Cypridopsinae Kaufmann, 1900 Genus *Potamocypris* Brady, 1870

Potamocypris meissneri sp. nov. http://zoobank.org/03D4496-F0A1-4C66-B481-BFDE1F939B4D Figures 2–4

Material examined. Type locality: SOUTH AFRICA, North-West Province, small temporary open pan (SA-9) near the village of Ganalaagte (Fig. 1, Suppl. material 1: Fig. 1A); 26°26'45"S, 25°32'19"E, elevation 1380 m a.s.l.; 1 Apr. 2011; T. Namiotko leg.



Figure 2. Carapace and valves of *Potamocypris meissneri* sp. nov. \bigcirc **A** RBINS INV.159060 **B** RBINS INV.159062 **C** RBINS INV.159061 **D** RBINS INV.159063 **E–G** RBINS INV.159060 **H–K** RBINS INV.159059. **A** carapace right view **B** carapace left view **C** carapace dorsal view **D** carapace ventral view **E** carapace right view of posterior end, detail of A **F** detail of external surface of A **G** carapace right view of anterior end, detail of A **H** left valve internal view **I** right valve internal view **J** left valve internal view of posterior part, detail of H **K** left valve internal view of anterior part, detail of H **K** left valve internal view of anterior end. (**A–D, H, I**); 200 µm (**E, G, J, K**); 50 µm (**F**); arrows indicate anterior end.

Holotype: • 1 \bigcirc (adult); dissected female stored on a permanent microscopic slide and valves stored dry on a micropalaeontological slide (RBINS INV.159058). **Paratypes:** SOUTH AFRICA • 2 \heartsuit (adults); same collection data as for holotype (OC-UG 110401-9A2L and OC-UG 110401-9A3L) • 136 \heartsuit (adults), 78 juv.; same collection data as for holotype: 115 \heartsuit and 78 juv. preserved in 96% ethanol; 16 \heartsuit stored as the holotype; 5 \heartsuit stored with carapaces stored on micropalaeontological slides (RBINS INV.159059–INV.159063); repositories: RBINS and OC-UG. **Accompanying ostracod fauna:** *Hemicypris* cf. *inversa* (Daday, 1913); *Limnocythere* cf. *stationis* Vávra, 1891.

Etymology. This species is named after Dr Włodzimierz Meissner, Professor of ornithology at the University of Gdansk, Poland, a long-standing friend of TN who provided unrelenting support in the collection of ostracods from all over the world and who has encouraged and helped TN to join various scientific expeditions for collecting ostracods.

Diagnosis. Carapace in lateral view somewhat ovoid, broadly rounded dorsally, with both extremities more or less equally rounded, ventral margin weakly concave, and maximum height situated at mid-length. Valves distinctly asymmetrical, with LV overlapping RV anteriorly and posteriorly, RV overlapping LV dorsally and ventrally. Anterior and posterior margins on LV with marginal flange, anterior one larger than posterior one. Carapace external surface hirsute, strongly ornamented with ridges, set with thickly rimmed pores with long sensilla. Antenna with long swimming setae. Terminal segment of maxillula palp spatulate with five claws. T1 with two hirsute branchial rays. CR of whip-like shape with elongated base, fused with distal long flagellum-like seta and set with additional short subapical seta.

Description. Female. Cp in lateral view (Fig. 2A, B) with posterior extremity more broadly rounded than anterior one, dorsal margin broadly rounded, ventral margin weakly concave, almost straight. Maximum height situated at mid-length. Valves distinctly asymmetrical, with LV overlapping RV along anterior and posterior margins with flanges (Fig. 2E, G). Carapace of hirsute appearance with reticulate external surface bearing numerous thickly rimmed normal pores with long sensilla (Fig. 2F). Cp in dorsal (Fig. 2C) and ventral view (Fig. 2D) with posterior extremity slightly rounded, anterior extremity more pointed. Greatest width situated slightly behind mid-length. RV slightly overlapping LV dorsally and ventrally. LV in internal view (Fig. 2H) subtriangular, with greatest height situated in front of mid-length, posterior part of dorsal margin straight and sloping towards the posterior side; anterior margin rounded, posterior margin almost straight, ventral margin slightly sinuous at mid-length. Anterior and posterior margins with marginal flanges, extending beyond inwardly displayed selvage along anterior and posterior margins, but peripheral along ventral margin (Fig. 2H, J-K). Flanges particularly expanded in the lower two-thirds of the anterior and posterior margins, with LV overlapping RV (Fig. 2A). Anterior and posterior calcified inner lamella narrow with one inner list, the latter not reaching halfway posterior margin. RV in internal view (Fig. 2I) ovoid, with maximum height situated in front of mid-length, anterior margin rounded, posterior



Figure 3. Limbs of *Potamocypris meissneri* sp. nov. \mathcal{Q} . Holotype (OC-UG 110401-9A1L) **A** second antenna **B** first antenna **C** mandibular palp **D** mandibular coxa. Scale bars: 100 µm. Abbreviation: ns = natatory setae.

margin less so. Anterior calcified inner lamella wide without inner list, but with submarginal peripheral selvage.

A1 (Fig. 3B) 7-segmented. First segment with one short subapical dorsal seta (not reaching tip of segment) and two long ventral setae. Second segment subquadrate with one short antero-dorsal seta. Rome organ not seen. Third segment $\sim 2 \times$ as long as wide, with two setae, one short antero-dorsal (reaching tip of next segment) and one very short antero-ventral seta. Fourth segment with two long antero-dorsal setae and one short antero-ventral seta (reaching 1/3 of next segment). Fifth segment bearing two long antero-dorsal setae and two ventral setae, one long and one of medium length (reaching beyond tip of terminal segment). Penultimate segment with four long apical setae. Terminal segment distally with three (two long and one medium-length) setae and an aesthetasc ya, length of aesthetasc ya $\sim 5/6$ of that of medium seta.

Chaetotaxic formula: I: A-1s, P-2l / II: A-1s / III: A-1s, P-1s / IV: A-2l, P-1s / V: A-2l, P-1l-1m / VI: A-4l / VII: D: 2l-1m-ya.

A2 (Fig. 3A) with protopodite, exopodite and 3-segmented endopodite. Basal segment of protopodite with two short ventro-apical setae. Second segment of protopodite with one long apical seta, reaching beyond first endopodal segment. Exopodite reduced to a small plate with three setae, two short and one long, the latter reaching halfway second endopodal segment. Endopodite 3-segmented. First endopodal segment with one long ventro-apical seta, extending beyond tip of terminal segment and one aesthetasc Y of medium length, divided in three parts, distal sensorial part with conspicuously sunken appearance; antero-dorsal with five long natatory setae (reaching tips of terminal claws) and one shorter (6th) seta reaching half of next segment. Second endopodal segment undivided, with two subequal medio-dorsal setae and four medio-ventral setae (t1-t4), two long, one medium and one short; distally with three z-setae, z1 and z3 long, z2 - 1/2 the length of z1 and z3, and three long serrated G-claws: G1 thick and apically strongly serrated, G2 and G3 more slender. Terminal endopodal segment subquadrate, with a long serrated claw GM, a shorter (~ 2/3 length of GM) smooth claw Gm and an aesthetasc y3 fused with slightly longer accompanying seta. Aesthetascs y1, y2 and seta g not seen, the latter almost certainly absent as typical of the subfamily.

Chaetotaxic formula: Pr: 11 / Exo: 11-2s / EI: A-51-1m, P: Y-11 / EII+III: A-2m, P-1m(t1)-2l(t2,3)-1s(t4), D-2l(z1,z3)-1m(z2)-3l(G1,2,3: ser) / EIV: 1l(GM: ser)-1m(Gm)-y3-1m

Md with sclerotised coxa (Fig. 3D) and 4-segmented palp (Fig. 3C). First palpsegment ventro-apically with two long plumed setae (S1 and S2), one long slender and smooth seta and a short but stout, smooth α -seta. Second segment antero-dorsally with two long slender and smooth setae and one thick plumed seta; ventrally with three unequally long smooth setae, one long hirsute seta and a stout and hirsute β -seta. Third segment antero-ventrally with one long hirsute seta and one short smooth seta; medio-dorsally with four setae, two reaching tip of terminal segment, and two longer setae, one of these smooth γ -seta; antero-dorsally with four setae reaching beyond tips of terminal segment. Terminal segment with four claws, two – 3× as long as length



Figure 4. Limbs of *Potamocypris meissneri* sp. nov. ♀ **A**, **B**, **C** holotype (OC-UG 110401-9A1L) **D** paratype (OC-UG 110401-9A3L) **E** paratype (OC-UG 110401-9A2L) **A** second thoracopod (walking leg) **B** maxillula **C** caudal ramus **D** first thoracopod (maxilliped) **E** third thoracopod (cleaning leg). Scale bars: 100 µm.

of terminal segment, one of these serrated, and two shorter claws. Md coxa typically elongated, distally with rows of teeth and small setae, and with one short smooth seta situated near the insertion place of the palp.

Chaetotaxic formula: Palp: I: In-1s(alfa)-1l-2l(S1,S2: pl) / II: In-1s(beta: pl)-1l(pl)-2m-1l, Ex:1l(pl)-2l / III: In-1l(pl)-1s, D-3m-1m(gamma), Ex-4m / IV: 2m-1l-1l(ser)

Mx1 (Fig. 4B) with three endites (with chaetotaxy incompletely illustrated), a 2-segmented palp and a large respiratory plate (not illustrated). Third endite with two smooth teeth bristles (Zahnborsten). First palp-segment dorso-apically with four unequal setae; medio-dorsally with one long, subapical seta, reaching beyond tip of terminal segment. Second palp-segment spatulate, apically with four stout claws (~ $1.5 \times$ as long as terminal segment) and one shorter claw (~ 1/2 length of others).

Chaetotaxic formula: Palp: I: Ex-3s-1l, In-1m / II: D-5s

T1 (Fig. 4D). Protopodite with two short a-setae; b-, c-, and d-setae absent, endite distally with a dozen apical setae (exact number not determined). Endopod elongated, apically with one long seta (h2) and two shorter setae (h1 and h3) of differing lengths. Respiratory plate with two hirsute rays.

Chaetotaxic formula: Pr: A-2s(a and a') / Mastic: D-? / Exo: 2m(pl) / E: D-3l

T2 (Fig. 4A) a walking limb. Protopodite with seta d2 of medium length, seta d1 absent. First three endopodal segments each with one ventro-apical seta. Setae e and f reaching tip of the next segment, seta g $\sim 3 \times$ as long as terminal segment. Fourth segment with one short seta (h3), one medium seta of length (h1) and a long claw, distally serrated (h2), the latter $\sim 3 \times$ as long as the second endopodal segment.

Chaetotaxic formula: Pr: A-1s(d2) / EI: A-1m / EII: A-1m / EIII: A-1m / EIV: P-1s(h3), D-1m(h1)-1l(h2 G:ser)

T3 (Fig. 4E) a cleaning limb. First segment with two long apical setae (dp and d2) and one shorter medio-ventral seta (d1). Second segment fused with third segment, with e-seta of medium length, and with f-seta reaching tip of segment. Distal part of limb consisting of a pincer-organ (fusion between tip of third segment and fourth segment), bearing a seta (h3) of medium length and a short, serrated seta (h2); h3 ~ 2/3 of the length of penultimate segment, h2 ~ 1/3 of length of h3.

Chaetotaxic formula: Pr: A-1l(d2)-1m(d1), P-1l(dp) / EI: A-1m(e) / EII + III: A-1s(f) / EIV: 1s(h2: ser)-1m(h3)

CR (Fig. 4C). Reduced to a whip-like structure, with elongated base fused with long flagellum-like seta and bearing a short subapical seta.

Remark. It should be noted that juveniles of this species do not have tubercles on the valves (see discussion).

Measurements (in µm). Cp (n = 4): L = 512–526, H = 305–306; LV (n = 11): L = 510–530, H = 278–298; RV (n = 11): L = 498–517, H = 301–319.

Male unknown.

Ecology. *Potamocypis meissneri* was collected only from the type locality in the North-West Province of South Africa. This is an open temporary pan with the following physical and chemical water properties: pH = 7.0, electrical conductivity = 36 μ S/cm and water temperature 25.8°C.

Key to southern African Potamocypris species (partly based on Martens 2001):

1	Natatory setae of A2 short (not reaching tips of terminal claws
	<i>P. paludum</i> Gauthier, 1939
_	Natatory setae of A2 long
2	Cp elongated ($L \ge 2 \times H$), crescent-shaped
_	Cp compressed (L < 2× H), differently shaped, not crescent-shaped 3
3	Cp subtriangular, dorsally arched with blunt angle P. gibbula (Sars, 1924)
_	Cp with dorsal margin broadly rounded or straight on a long distance and
	sloping down to the posterior4
4	Cp with posterior margin rounded; maximum height at mid-length
	P. meissneri sp. nov.
_	Cp with posterior margin straight; maximum height in front of mid-length5
5	RV with wide dorsal overlap of LV P. deflexa (Sars, 1924)
_	Dorsal overlap of RV minute or lacking P. humilis (Sars, 1924)

Genus Sarscypridopsis McKenzie, 1977

Sarscypridopsis harundineti sp. nov.

http://zoobank.org/45D19D9C-FD3E-4D1E-8C2C-65F14004373F Figures 5–7

Material examined. Type locality: BOTSWANA, North-West District, floodplains south of Okavango Delta (SA-103); grassy shore of seasonal pond near the city of Maun (Fig. 1, Suppl. material 1: Fig. 1B), 19°52'12"S, 23°20'23"E, elevation ca. 940 m a.s.l.; 15 Sept. 2012; T. Namiotko leg.

Holotype: • 1 \bigcirc (adult); dissected female stored on a permanent microscopic slide and valves stored dry on a micropalaeontological slide (RBINS INV.159064). **Paratypes:** BOTSWANA • 27 $\bigcirc \bigcirc$ (adults); same data as for holotype; 2 $\bigcirc \bigcirc$ stored as the holotype (OC-UG 120915-3A2L, 120915-3A3L); 22 $\bigcirc \bigcirc$ preserved in 96% ethanol (120915-30); 3 $\bigcirc \bigcirc$ stored on micropalaeontological slides (RBINS INV.159065– INV.159067); repositories: RBINS and OC-UG. **Accompanying ostracod fauna:** *Heterocypris oblonga* (Sars, 1924); *Limnocythere* cf. *stationis*; *Plesiocypridopsis newtoni* (Brady and Robertson 1870).

Additional material. BOTSWANA – North-West District: • SA-96 (Fig. 1, Suppl. material 1: Fig. 1C): 1 juv.; endorheic Lake Ngami; 20°28'57"S, 22°42'08"E; elevation ca. 930 m a.s.l.; 12 Sept. 2012; accompanying ostracod fauna: *Hemicypris inversa*; *Heterocypris giesbrechti* (G.W. Müller, 1898) • SA-97 (Fig. 1, Suppl. material 1: Fig. 1D): 11 QQ and 1 juv.; Thamalakane river near the city of Maun; 19°55'52"S, 23°30'38"E; elevation ca. 940 m a.s.l.; 13 Sept. 2012; accompanying ostracod fauna: *Candonopsis navicula* Daday, 1910; *Chrissia perarmata* (Brady, 1904); *Heterocypris oblonga; Isocypris* cf. priomena G.W. Müller, 1908; *Limnocythere* cf. stationis; *Physocypria* cf. capensis



Figure 5. Carapace and valves of *Sarscypridopsis harundineti* sp. nov. \bigcirc **A**, **B** RBINS INV.159066 **C** RBINS INV.159067 **D**, **E**, **F** RBINS INV.159065 **G**, **H** RBINS INV.159067 **A** carapace left view **B** detail of external surface of A **C** carapace ventral view **D** carapace dorsal view **E** carapace posterior part, detail of D **F** carapace anterior part, detail of D **G** carapace anterior part, detail of C **H** carapace posterior part, detail of C **I** left valve external view **J** right valve external view Scale bars: 400 µm (**A**, **C**, **D**); 50 µm (**B**); 200 µm (**E**–**H**); 100 µm (**I**, **J**); arrows indicate anterior end.

(Sars, 1895); Potamocypris mastigophora (Methuen, 1910); Sarscypridopsis cf. elizabethae (Sars, 1924); Sclerocypris sp., Stenocypris malayica Victor & Fernando, 1981; Strandesia cf. prava Klie, 1935 • SA-98 (Fig. 1, Suppl. material 1: Fig. 1E): $6 \ Q \ Q$; floodplains south of Okavango Delta, temporary channel near the city of Maun; 19°52'15"S, 23°21'06"E; elevation ca. 940 m a.s.l.; 14 Sept. 2012; accompanying ostracod fauna: *Heterocypris giesbrechti* • **SA-99** (Fig. 1, Suppl. material 1: Fig. 1F): 16 \bigcirc and 1 juv.; floodplains south of Okavango Delta, temporary channel near the city of Maun; 19°52'15"S, 23°20'45"E; elevation ca. 940 m a.s.l.; 14 Sept. 2012; accompanying ostracod fauna: Heterocypris oblonga; Potamocypris deflexa (Sars, 1924); Potamocypris mastigophora; Zonocypris costata (Vávra, 1897) • SA-100 (Fig. 1, Suppl. material 1: Fig. 1G): 11 \bigcirc ; floodplains south of Okavango Delta, flooded swamp and grassland near the city of Maun; 19°52'04"S, 23°20'38"E; elevation ca. 940 m a.s.l.; 14 Sept. 2012; accompanying ostracod fauna: Heterocypris oblonga; Stenocypris malayica; Zonocypris tuberosa G.W. Müller, 1908 • SA-101 (Fig. 1, Suppl. material 1: Fig. 1H): 6 $\mathbb{Q}\mathbb{Q}$; floodplains south of Okavango Delta, isolated pool in flooded grassland near the city of Maun; 19°51'39"S, 23°19'41"E; elevation ca. 940 m a.s.l.; 15 Sept. 2012; accompanying ostracod fauna: Heterocypris oblonga • SA-102 (Fig. 1, Suppl. material 1: Fig. 11): $1 \stackrel{\bigcirc}{_{\sim}}$; floodplains south of Okavango Delta, floodplain channel near the city of Maun; 19°52'06"S, 23°20'41"E; elevation ca. 940 m a.s.l.; 15 Sept. 2012; accompanying ostracod fauna: Heterocypris oblonga.

All individuals collected by T. Namiotko; 51 \bigcirc and 3 juv. are stored in 96% ethanol and 3 \bigcirc are stored as holotype. Repositories: RBINS and OC-UG.

Etymology. This species is named after the term "reed-bed" (Latin: *harundine-tum*), the original meaning of the name of the town Maun in Botswana close to the sites from where *Sarscypridopsis harundineti* was collected. The name Maun is derived from the language of Bantu-speaking people and translates as "the place of river reeds".

Diagnosis. Carapace in lateral view with anterior and posterior margins nearly symmetrically rounded, dorsal margin almost evenly rounded with greatest height situated just behind mid-length, ventral margin almost straight. RV overlapping LV anteriorly, posteriorly and ventrally, LV slightly overlapping RV dorsally. Carapace surface smooth (with fine reticulation in the central area), with rare thickly rimmed normal pores with short sensilla, situated mostly in the posterior and postero-dorsal parts. Antenna with long swimming setae, and supporting aesthetasc Y with distinctive distal bulbous sensory part. Terminal segment of maxillular palp elongated, ~ 2× as long as wide, bearing four long claws. T1 with four branchial rays. CR reduced, with elongated, triangular base.

Description. Female. Cp in left lateral view (Fig. 5A) with anterior and posterior margins nearly symmetrically rounded, dorsal margin almost evenly rounded, with greatest height situated just behind mid-length; ventral margin almost straight. RV overlapping LV anteriorly and posteriorly. LV slightly overlapping RV dorsally, RV overlapping LV ventrally (Fig. 5E–H). External surface smooth with fine reticulation in the central area; with rare, thickly rimmed pores with extending sensilla situated mostly



Figure 6. Limbs of *Sarscypridopsis harundineti* sp. nov. *Q*. **A, B, E, F** holotype (OC-UG 120915-3A1L) **C, D** paratype (OC-UG 120915-3A3L) **A** Second antenna **B** First antenna **C** Mandibular palp **D** Maxillula **E** Mandibular coxa **F** Maxillular respiratory plate. Scale bars: 100 μm. Abbreviation: ns = natatory setae.

in the anterior and postero-dorsal parts (Fig. 5B). Cp in dorsal (Fig. 5D) and ventral views (Fig. 5C) sub-elliptical, lateral margins unevenly rounded, widening posteriorly; posterior edge broadly rounded, anterior one more pointed. Greatest width situated behind mid-length. LV in internal view (Fig. 5I) ovoid, with greatest height situated at mid-length. Anterior and posterior margins almost equally rounded, ventral margin slightly sinuous at mid-length. Anterior and posterior and posterior calcified inner lamella narrow with marginal selvage. RV in internal view (Fig. 5J) with posterior margin broadly rounded, anterior margin more pointed and with ventral margin almost straight. Anterior and posterior calcified inner lamella wider than on LV; no selvage or inner list.

A1 (Fig. 6B) 7-segmented. First segment with one short subapical dorsal seta (reaching beyond tip of segment) and two long ventro-apical setae. Second segment with one short dorso-apical seta (reaching 1/3 of length of next segment) and a large ventral Rome organ. Third segment ~ $0.5 \times$ as long as wide, with two apical setae, one short dorsal seta (reaching beyond tip of next segment) and one short ventral seta (not reaching tip of next segment). Fourth segment with two long dorso-apical setae and one short ventro-apical seta (reaching 1/3 of penultimate segment). Fifth segment bearing two long ventro-apical setae and two dorso-apical setae, one long and one short (the latter nearly reaching tip of terminal segment). Penultimate segment with four long apical setae. Terminal segment with three (two long and one medium) setae and one aesthetasc ya, the latter slightly longer than the seta of medium length.

Chaetotaxic formula: I: A-1s, P-2l / II: A-1s, P-r / III: A-1s, P-1s / IV: A-2l, P-1s / V: A-2l, P-1l-1s / VI: A-4l / VII: D: 2l-1m-ya.

A2 (Fig. 6A) with protopodite, exopodite and 3-segmented endopodite. Basal segment of protopodite with two short ventro-apical setae. Second segment of protopodite with one long ventro-apical seta. Exopodite reduced to a small plate with three setae, two short and one long. Endopodite 3-segmented. First endopodal segment with long ventro-apical seta, extending beyond tip of terminal segment; a large aesthetasc Y with a distinct distal bulbous sensory part; dorso-apically with five long natatory setae (reaching far beyond tips of terminal claws) and one shorter (6th) seta reaching 1/3 of length of next segment. Second endopodal segment undivided, medio-dorsally with two subequally long setae and medio-ventrally with four unequal setae (t1-t4), one long and three short; distally with three long z-setae (z1, z2, z3) and three long serrated G-claws: G2 thick and apically strongly serrated, G1 and G3 more slender. Terminal (third) endopodal segment subquadrate with a long serrated claw GM, a shorter (~ 1/2 length of GM) smooth claw Gm and an aesthetasc y3 fused with slightly longer accompanying seta. Aesthetascs y1, y2 and seta g not seen, the latter almost certainly absent as typical of the subfamily.

Chaetotaxic formula: Pr: 11 / Exo: 11-2s / EI: A-51-1s, P-Y-11 / EII+III: A-2l, P-1s(t1)-11(t2)-2s(t3,4), D-31(z1,z2,z3)-31(G1,2,3: ser) / EIV: 11(GM: ser)-1m(Gm)-y3-1s

Md with sclerotised coxa (Fig. 6E) and 4-segmented palp (Fig. 6C). Md-coxa elongated, distally with rows of teeth and small setae, and with one short, smooth seta situated near the palp. First palp-segment ventro-apically with two long plumed setae (S1 and S2), one long, slender seta and a short smooth α -seta, situated in between the two S-setae. Second segment dorso-apically with three unequally long slender setae; ventrally with three long, smooth setae, one medium hirsute seta and hirsute, cone-shaped β -seta. Third segment ventro-apically with one long and one short seta; medio-apically with three setae, all reaching tip of terminal segment and a hirsute and long γ -seta; dorso-apically with four setae, all extending far beyond tips of terminal segment. Terminal segment bearing four claws, three ~ 4× as long as terminal segment and one shorter.

Chaetotaxic formula: Palp: I: In-1s(alfa)-11-21(S1,S2: pl) / II: In-1s(beta: pl)-1m(pl)-3l, Ex-3l / III: In-11-1s, D-3l-11(gamma: pl), Ex-4l / IV: 3l-1m

Rake-like organs (food-rakes) (Fig. 7A) T-shaped, each with nine apical teeth.

Mx1with three endites and 2-segmented palp (Fig. 6D) and a large respiratory plate (Fig. 6F). First endite with two short setae near its base. Third endite with two smooth teeth bristles. First palp-segment with four unequal dorso-apical setae, one long, one medium and two short; medio-apically with one seta (reaching 1/2 length of terminal segment). Second segment elongated (~ 2× as long as wide), apically with four unequal but long claws (~ 2× as long as terminal segment). Respiratory plate large and elongate, distally with a row of more than eleven smooth rays and three long plumose rays.

Chaetotaxic formula: Palp: I: Ex-2s-1m-1l, In-1s / II: D-4m

T1 (Fig. 7B). Protopodite with two short unequal a-setae; b-, c- and d-setae absent, endite distally with a dozen apical setae (exact number not determined). Endopod elongated, apically with one long seta (h2) and two shorter setae (h1 and h3). Respiratory plate with four long rays with swollen bases.

Chaetotaxic formula: Pr: A-2s(a and a') / Mastic: D-? / Exo: 4l / E: D-3l

T2 (Fig. 7C, D) a walking limb. Protopodite with seta d2 of medium length, seta d1 absent. First two endopodal segments with one long antero-apical seta each. Seta e reaching half of the penultimate segment and seta f reaching tip of terminal segment. Third endopodal segment with two antero-apical setae, one medium length g-seta and one very short seta. Fourth segment with one very short seta (h3), one short seta (h1) and long, strongly curved and serrated claw (h2); distal claw ~ 3× as long as the second endopodal segment.

Chaetotaxic formula: Pr: A-1m(d2) / EI: A-11 / EII: A-11 / EIII: A-1m-1s / EIV: P-1s(h3), D-1s(h1)-11(h2 G:ser)

T3 (Fig. 7E) a cleaning limb. Protopodite with two long setae (dp and d2) and one shorter seta (d1). First endopodal segment with short subapical e-seta. Second and third endopodal segments fused, with short f-seta not reaching tip of segment. Terminal part (fusion between distal part of third and fourth segment) a pincer organ, bearing a medium length seta (h3), a short serrated seta (h2), and a very short seta (h1), length of seta h3 \sim 2/3 that of fused segment, seta h2 \sim 1/2 length of seta h3.

Chaetotaxic formula: Pr: A-11(d2)-1m(d1), P-11(dp) / EI: A-1s(e) / EII + III: A-1s(f) / EIV: 1s(h2: ser)-1m(h3)

CR (Fig. 7F). Reduced, with elongated, triangular base; distally with long flagellum-like seta and subapically with a short seta.

Measurements (in μ m). Cp (n = 3): L = 433–464, H = 259–282; LV (n = 6): L = 430–461, H = 250–272; RV (n = 6): L = 444–473, H = 261–275.



Figure 7. Limbs of *Sarscypridopsis harundineti* sp. nov. \bigcirc **A, B, C, F** paratype (OC-UG 120915-3A3L) **D** paratype (OC-UG 120915-3A2L) **E** holotype (OC-UG 120915-3A1L) **A** Food-rake **B** First thoracopod (maxilliped) **C** Second thoracopod (walking leg) **D** Second thoracopod distal end **E** Third thoracopod (cleaning leg) **F** Caudal ramus. Scale bars: 100 µm.
Male unknown.

Ecology. Sarscypridopsis harundineti was found in eight temporary waterbodies of the vast floodplains south of the Okavango Delta in northern Botswana. Habitats include both lotic (river side channel, floodplain channel) and lentic waters (flooded swamp, grassland, isolated pool) as well as the endorheic Lake Ngami. The species occurred at the pH range of 6.5–7.7, the electrical conductivity range of 102–464 μ S/cm, and the water temperature range of 19.8–33.7 °C.

Discussion

Potamocypris and Cyprilla Sars, 1924

Sars (1924a) described the genus *Cyprilla*, based on the carapace shape and presence of large flanges on the left valve, causing a noticeable LV > RV overlap. Five species were assigned to this genus, all raised from dry mud or obtained from water samples, from South Africa: C. arcuata Sars, 1924, C. deflexa Sars, 1924, C. gibbula Sars, 1924, C. humilis Sars, 1924 and C. producta Sars, 1924. In the same publication, Sars (1924a) noticed that Cyprilla differs from Potamocypris "in the general appearance of the shell and in the mutual relation of the valves, as also apparently in the sculpture", but shares with Potamocypris reduced caudal rami and a spatulate terminal segment of the Mx1 palp. According to Gauthier (1939), these features cannot be considered diagnostic, and thus he transferred the five Cyprilla species to Potamocypris, automatically synonymising *Cyprilla* with *Potamocypris*. The same view was supported by Meisch (1984, 1985) and George and Martens (2002). While redescribing P. humilis from the Outer Hebrides off the west coast of Scotland, Horne and Smith (2004) noticed prominent tubercules on juvenile carapaces, the trait not yet described in any Potamocypris. They partly agreed with Purasjoki (1948) who considered the presence of tubercules in juveniles a *Cyprilla* trait, in this way questioning the previously proposed synonymisation of the two genera. This merits a further study, but we confirm that none of the juveniles of P. meissneri we collected had tubercules. Therefore, we feel confident in describing the new species as a member of *Potamocypris*.

Potamocypris meissneri differs from other species of the genus by the presence of 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. Out of five southern African Potamocypris species only one, P. paludum Gauthier, 1939 (nom. nov. pro Cyprilla arcuata Sars, 1924 nec Sars, 1903 – fide Gauthier, 1939), has short swimming setae on the second antennae, which clearly distinguishes it from the new species described here. Two further species, P. mastigophora (of which Cyprilla producta Sars, 1924 is a synonym – fide McKenzie, 1971) and P. gibbula have different carapace shapes from P. meissneri: more elongated and crescent-shaped in P. mastigophora, and more subtriangular, and dorsally arched with a blunt angle in P. gibbula. The remaining two species (i.e., P. humilis and P. deflexa) are more similar to *Potamocypris meissneri* as they also have ornamented carapaces. However, unlike in *Potamocypris meissneri*, the posterior part of the dorsal margin in the two species is almost straight, sloping down and making a distinct angle with the characteristically truncated and almost straight posterior margin. In addition, none of the presently known species of *Potamocypris* has this type of pronounced external valve ornamentation. *Potamocypris narayanani* George & Martens, 2002 carries conspicuous stiff setae and has a pitted valve surface, but lacks the prominent ridges and has a huge dorsal hump on the left valve.

Sarscypridopsis

The originally assigned type species, *S. gregaria* (Sars, 1895), was placed into the synonymy of *S. aculeata* (Costa, 1847) by Sywula (1966), and both were originally assigned to the genus *Cypridopsis*, which originally included all cypridinid ostracods with a flagellate CR. However, over time, as more species with this trait had been found, new genera were distinguished, *Sarscypridopsis* being one of those. McKenzie (1977) ignored the synonymy proposed by Sywula (1966) and made *S. gregaria* the type species of his genus *Sarscypridopsis*. The status of *S. gregaria* has to be rechecked on type material, to clearly determine which is the true type species of this genus: *S. aculeata* with *S. gregaria* being its junior synonym, or *S. gregaria* as a valid species. Nominally, of course, *S. gregaria* will always remain the designated type species.

According to McKenzie (1977) and Meisch (2000) *Sarscypridopsis* is diagnosed by the following morphological characters: carapace rather small (< 0.9 mm), subtriangular in shape with smooth or pitted surface; RV overlapping LV ventrally, anteriorly and posteriorly; calcified inner lamella broad anteriorly and narrower posteriorly; distal segment of Mx1 palp cylindrical and ramus of the CR triangular. As our new species show all of these characteristics, we herewith assign it to the genus *Sarscypridopsis*. However, compared to other congeners, the base of the CR is unusually elongated.

Sarscypridopsis is mostly an Afrotropical genus (Meisch et al. 2019). Of the 17 species presently assigned to this genus, only three are known to occur also outside the African continent. *Sarscypridopsis aculeata* is nearly cosmopolitan (Meisch et al. 2019), *S. lanzarotensis* (Mallwitz, 1984) has been found in Spain including Canary Islands (Mallwitz 1984; Meisch 2000; Castillo-Escrivà et al. 2016), Italy (Pieri et al. 2015), and Morocco (Scharf and Meisch 2014), while *S. ochracea* (Sars, 1924), except for the Afrotropical region, has also been reported from the Oriental and Australian regions (Meisch et al. 2019), although these latter identifications seem unlikely from a zoogeographical point of view and need confirmation.

Most of the *Sarscypridopsis* species are poorly described and only for the following three species the morphology of the soft parts is (partly) known: *S. aculeata, S. katesae* (Hartmann, 1957) and *S. lanzarotensis*. In all three of these species, the respiratory plate of T1 carries five rays, while *S. harundineti* has only four rays, although admittedly this character is often very difficult to observe. Another difference between the new species and *S. aculeata* and *S. lanzarotensis* is the presence of two smooth teeth

bristles on the third endite of Mx1 in *S. harundineti*. In the other two species the proximal bristle is serrated as is the neighboring one (Mallwitz 1984; Meisch 2000). In *Sarscypridopsis katesae* these teeth bristles are also smooth (Hartmann 1957).

Despite of the lack of comparative characters, *Sarscypridopsis harundineti* can be easily distinguished from its South African congeners by the unique, more rounded valves shape, and the smaller carapace (L = 0.43–0.47 mm versus 0.54–0.80 mm). The greatest height is situated just behind mid-length in the new species, unlike in *S. clavata* (Sars, 1924), *S. echinata* (G.W. Müller, 1908), *S. elizabethae*, *S. hirsuta* (Sars, 1924), *S. punctata* (Sars, 1924), *S. reniformis* (Sars, 1924) and *S. striolata* (Sars, 1924) in which the dorsal margin of the carapace is more arched and the greatest height is situated more to the front. *Sarscypridopsis ochracea* (Sars, 1924), *S. pyramidata* (Sars, 1924), *S. tonsa* (Sars, 1924) and *S. trigonella* (Sars, 1924) have sub-triangular carapaces, while *S. glabrata* (Sars, 1924) has more elongated one. The carapace size and the shape in the lateral view of *Sarscypridopsis harundineti* is most similar to *S. brevis* (Sars, 1924) and *S. sarsi* (Klie, 1935). The former can be distinguished by a very hirsute external surface of the carapace, while the latter has a distinctly globular carapace in dorsal view (Sars 1924a, 1924b).

Taking into account the mentioned gaps in information on taxonomic traits, we conclude that southern African Cypridopsinae, especially representatives of the genus *Sarscypridopsis*, urgently need integrated taxonomic revision, i.e., by means of both morphological characters (including redescriptions based on both the type and newly collected material) and DNA-sequence data.

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Supplementary material I

Pictures of the sites from where new species *Potamocypris meissneri* sp. nov. and *Sarscypridopsis harundineti* sp. nov. were collected

Authors: A. Szwarc, K. Martens, T. Namiotko

Data type: Images

- Explanation note: Pictures of the sites from where *Potamocypris meissneri* sp. nov. and *Sarscypridopsis harundineti* sp. nov. were collected in the North-West Province of South Africa (A) and in the floodplains south of the Okavango Delta near the town of Maun (except C) in the North-West District of Botswana (B-I), respectively. A SA-9: a small temporary open pan near the village of Ganalaagte B SA-103: grassy shore of a seasonal pond C SA-96: endorheic Lake Ngami D SA-97: Thamalakane river E SA-98: a temporary channel F SA-99: a temporary channel G SA-100: a flooded swamp and grassland H SA-101: an isolated pool in flooded grassland I SA-102: a floodplain channel.
- Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons.org/licenses/odbl/1.0/). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.
- Link: https://doi.org/10.3897/zookeys.1076.76123.suppl1





















Gdańsk, 5 czerwca 2023 r.

Oświadczenie

Oświadczam, że w pracy:

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mój wkład polegał na udziale w opracowaniu wspólnej koncepcji pracy, oznaczeniu materiału, wykonaniu trwałych preparatów osobników typowych, przygotowaniu treści manuskryptu, mapy terenu badawczego oraz rysunków części miękkich opisywanych gatunków i tablic ze zdjęciami skorupek spod skaningowego mikroskopu elektronowego.

a Scoarc

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Statement TO WHOM IT MAY CONCERN

Brussels, 05 06 2023

I hereby declare that my contribution to the paper:

Szwarc A., Martens K., Namiotko T. 2021. Two new Cypridopsinae Kaufmann, 1900 (Crustacea, Ostracoda) from southern Africa. *ZooKeys* 1076 (13): 83-107. https://doi.org/10.3897/zookeys.1076.76123

includes assistance with the taxonomic identification, providing access to the museum collection of the reference material and to the scanning electron microscope facility, as well as help in preparation of the manuscript.

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mój wkład polegał na zebraniu materiału badawczego, udziale w stworzeniu koncepcji pracy, wstępnym oznaczeniu taksonomicznym materiału oraz udziale w edycji tekstu.

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Article Biodiversity of Non-Marine Ostracoda (Crustacea) of Botswana: An Annotated Checklist with Notes on Distribution

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Abstract: Botswana constitutes a major gap in our knowledge of the distribution of Ostracoda in the region of Southern Africa, restraining thorough biogeographic interpretations. We combine records from previously published surveys along with our own field collections to provide a collation of living and fossil (Late Pleistocene to Holocene) Ostracoda recorded in Botswana. Our survey yielded 17 species, of which nine species have not been recorded before in the country. Including the present update, 54 species (45 living and nine fossil or subfossil) belonging to 22 genera of five families (with 76% species belonging to the family Cyprididae) are currently reported from Botswana. Yet, 23 taxa are left in open nomenclature, indicating the urgent need for sound systematic studies on harmonizing taxonomy of Southern African ostracods, especially of those inhabiting small temporary waterbodies, considered as threatened with extinction before being properly described or discovered. This updated checklist provides detailed information about the distribution and habitat of each recorded species. Species richness, distribution patterns, and diversity of ostracod species regionally and in different freshwater ecoregions are also discussed. We found low alpha (site) diversity (mean 3.3 species per site) and a significant difference in species composition and beta diversity of the Okavango ecoregion versus the Kalahari and Zambezian Lowveld ecoregions.

Keywords: Southern Africa; species catalogue; ostracods; taxonomic diversity; temporary waters

1. Introduction

Inland waters are home to remarkably high taxonomic diversity, but are also among the world's most endangered ecosystems in the world. Due to various forms of human activity (such as overexploitation, pollution, flow modification, and habitat degradation) and climate change, one-third of inland aquatic species are threatened with extinction and about 70% of wetlands have been lost since 1900 [1–3]. One of the most threatened types of inland aquatic ecosystems are temporary waterbodies, the number of which is disappearing at an alarming rate [4–7]. To assess the consequences of the anthropogenic stress and climate-change effects on aquatic biological diversity, as well as to evaluate the ecological status of inland wetlands and effectively manage them, it is essential to have comprehensive taxonomic and distributional inventories of aquatic organisms. The acquisition of such databases is considered one of the key topics for current research on freshwater biodiversity in most endangered regions [8].

Inland water ecosystems across much of the region of Southern Africa (hereafter defined as the region south of (and including) the catchments of the Cunene and Zambezi rivers) are under increasing pressure and suffer from both recent human activity and natural climate variation [9], while biodiversity data on their invertebrates are often lacking [10]. Here, we combine records from previously published surveys along with our own field collections to provide a collation of living and fossil (Late Pleistocene to Holocene) Ostracoda inhabiting the wetlands of Botswana.



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Botswana is a land-locked country with a prevailing warm, semi-arid climate where droughts are common and rainfall is unpredictable. Mean annual rainfall (restricted mainly to October-April) varies from over 650 mm in the north-east to less than 250 mm in the south-west of the country, and high evapotranspiration rates increase the aridity of the environment. These natural arid conditions and frequent drought periods make Botswana highly susceptible to desertification, further exacerbated by unsustainable grazing practices and soil erosion, which imperil already-stressed water resources and contribute to waterquality problems [11–13]. The wetlands of Botswana consist mainly of a variety of seasonally rain-fed waterbodies, such as temporary freshwater or salt pans, temporary rivers, and man-made reservoirs. The only perennial wetlands include the systems of the Okavango river and the Chobe-Linyati-Kwando rivers, both in the north of the country [12]. Botswana includes parts of seven freshwater ecoregions recognized by Abell et al. [14]; from the north to south these are: Upper Zambezi Floodplains, Okavango, Kalahari, Zambezian Lowveld, Southern Kalahari, Southern Temperate Highveld, and Middle Zambezi-Luangwa, of which only a very small part intrudes Botswana in the north-east. The xeric ecoregions of Kalahari and Southern Kalahari cover most of the country. All the above-mentioned types of wetlands, unevenly distributed throughout the freshwater ecoregions in Botswana, constitute habitats suitable for ostracods.

Ostracods are a class of bivalve microcrustaceans that occur in almost all aquatic habitats and have great potential as a model group in both fundamental and applied research, such as advanced ecotoxicological studies, monitoring anthropogenic disturbances, and predicting environmental impacts [15]. Despite a long history of Southern African nonmarine ostracod studies [16], checklists of Southern African non-marine ostracods [16,17] include only a few records from Botswana (listed mostly as from Kalahari) collected during the Schultze expedition to Namaland and Kalahari in 1903–1905 [18], the Vernay–Lang Kalahari Expedition in 1930 [19], and the Eccles Makarikari Expedition in 1957 [16]. Since these compilations, there have been no faunistically comprehensive works within the area of Botswana published, except some single-site (or small area) records e.g., [20–22].

To attempt to remedy this situation, and to encourage other authors to contribute towards an acquisition of a comprehensive distributional and taxonomic database on the Ostracoda of Botswana, we provide an updated checklist of the living and fossil (Late Pleistocene to Holocene) distribution of non-marine ostracods in this Southern African country, based on both the records published to date and on our own unpublished collection. The checklist does not, however, constitute a revision of previous studies, but rather a collation that may be used for further detailed taxonomic work upon re-examination of the published material. We also investigated patterns in taxonomic diversity at regional and local scales (gamma and alpha diversity), as well as regional differences in the taxonomic composition between some freshwater ecoregions and sites (beta diversity).

2. Materials and Methods

Although several papers mention ostracods from the wetlands of Botswana, mostly those containing records identified down to species level were used in the checklist. Citations of species names originally left in open nomenclature (sp., cf., sp. n., new sp. ?), however, are also included as these most probably represent new species awaiting formal description. Bibliographic searching was undertaken in several ways. First, much use was made of previously published checklists of living ostracods from inland waters of Southern Africa ([16,17]) and the manual or electronic checking of citation lists in published articles was undertaken by the authors. For finding relevant papers, we also used electronic-searching facilities and indices, i.e., the databases of Web of Sciences (All Databases) and Scopus, employing the following simple keyword combination: "Botswana" AND "ostracod*". We believe that the literature used is complete, though some abstracts, theses, or other papers not generally available may have been missed.

The checklist deals with the occurrence of each species as originally determined from published data; however, these records are placed within the context of presently acceptable

taxonomic conventions, and species are listed in a taxonomically-based order following [23]. Species with first records for Botswana are preceded by an asterisk (*).

Each species entry in the checklist includes Botswana records in chronological order (both historical and own) with the following data: the reference with the originally published species name, verbatim locality name with the original site/sample codes, geographical coordinates (in the DMS system, i.e., degrees minutes seconds), altitude (taken from the source reference or estimated on the original description of the locality using Google Earth), and freshwater ecoregion (FEOW) within which the locality is situated (according to the regionalization of the Freshwater Ecoregions Of the World in [14]). These data are followed by the habitat type, collection date, and details on the age, number, sex, and ontogenetic stage of the collected specimens, if available. Other sections under each species headings (excluding species left in open nomenclature) include global distribution after [23] (with the codes of the regions as follows: AT = A frotropical, AU = A ustralasian, NA = Nearctic, NT = Neotropical, OL = Oriental, PA = Palaearctic, PAC = Pacific Oceanic Islands) and distribution in Southern Africa, largely after [17,24] (with country codes as follows: BW = Botswana, LS = Lesotho, NAM = Namibia, ZA = South Africa, ZW = Zimbabwe). Finally, notes with taxonomic remarks or comments on the distribution (if relevant) close the species entry.

Our collection of living ostracods was made from 11 sites in Botswana between 11 and 20 September 2012. Samples were collected with a hand-net (mesh size of 120 μ m) sweeping the bottom surface at the depth of <50 cm and then fixed in 96% ethanol. In the laboratory, ostracods were sorted, identified, and counted using a stereoscopic microscope. The soft parts of ostracods were mounted in glycerine while valves were stored on the micropalaeon-tological slides following [25]. Specimens were identified down to the species level using specialized literature (e.g., [17,26–30]). The specimens are housed in the Ostracod Collection of the Department of Evolutionary Genetics and Biosystematics of the University of Gdansk, and the type specimens of *Sarscypridopsis harundineti* are deposited in the Collection of the Royal Belgian Institute of Natural Science, Brussels (RBINS INV.159064–159067).

The map of the sampling sites was made with ArcGIS Pro 2.8.3. Esri Inc. (Figure 1).

To evaluate if the sampling effort was sufficient to represent the biodiversity of the ostracod assemblages in the studied area, we performed species accumulation curves of the observed and expected species number by the Chao 2 index based on 28 sites with the living incidence records (including both historical and own material). The mean and standard deviation of species richness estimates were calculated from 9999 permutations with sites added in random order. To test for similarity in beta diversity among freshwater ecoregions (with the number of sites/samples >2), the test for homogeneity of multivariate dispersions (PERMDISP) was used with 9999 permutations and sites added in random order on the basis of a taxonomically dissimilarity measure Gamma+, which is a natural extension of the Bray–Curtis dissimilarity on presence/absence data. A five-level classification (species, genus, subfamily, family, and superfamily) was used according to [23]. We also tested for significance of species composition among freshwater ecoregions using a one-way ANOSIM followed by pair-wise tests. To visualize the pattern exhibited by the ecoregions on the basis of their ostracod fauna, a non-metric Multi-Dimensional Scaling (mMDS) ordination was constructed from bootstrap averages using the above Gamma+ resemblance matrix. All procedures were run on PRIMER 7 software [38] with the PERMANOVA+ add-on package [39].



Figure 1. Map of Botswana with the sampling sites. Historical records are indicated with orange triangles and letters (A–T), while our own records are indicated with yellow diamonds and numbers (1–11). A–H [22]: A—SA-96, B—SA-97, C—SA-98, D—SA-99, E—SA-100, F—SA-101, G—SA-102, H—SA-103; I–J, L [19]: I—Tsotsoroga, J—N'kate Pan, L—Kaotwe Pan; K [18,31]: Ku-Gudië; M [32,33]: Makgadikgadi Pan; N [34]: Makgadikgadi Pan; O [35,36]: Sua Pan "Kubu Island"; P–R [21]: P—North Basin of Makgadikgadi Pan, Q—South Basin of Makgadikgadi Pan, R—Middle Basin of Makgadikgadi Pan; S–T [20,37]: S—Kgale Siding, T—Thamaga. Own collection: 1—SA-95; 2—SA-107; 3—SA-108; 4—SA-109; 5—SA-110; 6—SA-112; 7—SA-113; 8—SA-114; 9—SA-115; 10—SA-116; 11—SA-117.

3. Results

3.1. Checklist of Non-Marine Living and Fossil (Late Pleistocene to Holocene) Ostracoda of Botswana

Class: Ostracoda Latreille, 1802 Subclass: Podocopa Sars, 1866 Order: Podocopida Sars, 1866 Suborder: Cypidocopina Baird, 1845 Superfamily: Cypridoidea Baird, 1845 Family: Cyprididae Baird, 1845 Subfamily: Cypricercinae McKenzie, 1971 Tribe: Cypricercini McKenzie, 1971 Genus: *Cypricercus* Sars, 1895

*Cypricercus cf. cuneatus Sars, 1895

Present paper: Kolobeng river near the village of Manyana, site no SA-113/coord. 24°46′08″ S, 25°35′22″ E, 1133 m a.s.l./FEOW: 576 Zambezian Lowveld/Habitat: river/Coll. date: 20 September 2012/2 ♀♀, 9 juv.

Cypricercus inermis (Brady, 1904)

Jocqué et al. [20]: Kgale Siding near Gabarone/coord. 24°40′30″ S, 25°50′20″ E, 1040 m a.s.l./FEOW: 576 Zambezian Lowveld/Habitat: nine temporary granite rock pools/Coll. dates: entire inundation cycle of the rainy season of 2002–2003/number of specimens unspecified.

Distribution: AT (BW, ZA)

Genus: Strandesia Stuhlmann, 1888

Strandesia cf. prava Klie, 1935

- Szwarc et al. [22]: Thamalakane river near the city of Maun, site no SA-97/coord. 19°55′52″ S, 23°30′38″ E, 940 m a.s.l./FEOW: 569 Okavango/Habitat: river side channel/Coll. date: 13 September 2012/2 ♀♀, 9 juv;
- Present paper: Botanic Garden in the city of Gabarone, site no SA-112/coord. 24°39′56″ S, 25°56′40″ E, 987 m a.s.l./FEOW: 576 Zambezian Lowveld/Habitat: artificial rockpool/Coll. date: 19 September 2012/2 ♀♀.

Strandesia n. sp. (gr. sudanica) Sywula, 1970

Jocqué et al. [20]: Kgale Siding near Gabarone/coord. 24°40'30" S, 25°50'20" E, 1040 m a.s.l./FEOW: 576 Zambezian Lowveld/Habitat: nine temporary granite rock pools/Coll. dates: entire inundation cycle of the rainy season of 2002–2003/number of specimens unspecified.

Strandesia sp.

Riedel et al. [36]: sediment core 1.5 km east of Kubu Island at the south-western edge of Sua Pan in Makgadikgadi Basin/coord. 20°53'30″ S, 25°50'30″ E, 900–903 m a.s.l./FEOW: 570 Kalahari/Habitat: palaeo-mega-lake system (sediment core to 3.0 m depth)/Coll. dates: September 2007, April 2008, July 2010/fossil valves in a sedimentary sequence dated from ca. 37 ka to 2 ka cal. BP (calibrated years before the present).

Subfamily: Cypridinae Baird, 1845 Genus: *Pseudocypris* Daday, 1910

Pseudocypris circularis Sars, 1924

- Barnard [19]: 1 mile north of Tsotsoroga Pan, localities nos 1204a and 1214/approx. coord. 18°43'00" S, 24°21'00" E, 940 m a.s.l./FEOW: 569 Okavango/Habitat: not indicated/Coll. dates: 19–20 June 1930/number of specimens unspecified;
- Barnard [19]: N'kate (= Nekati) Pan, locality no 1505/approx. coord. 20°05'00" S, 26°01'00" E, 920 m a.s.l./FEOW: 570 Kalahari/Habitat: shallow pan in limestone formation/Coll. date: 7 August 1930;
- McKenzie [16]: Makarikari expedition, no further details given/FEOW: 570 Kalahari/Habitat: not indicated/Coll. date: 2.10.1958/number of specimens unspecified, det. D.H. Eccles;
- Martens [24]: collected by the Vernay-Lang Kalahari expedition 1930, no further details given (probably same localities as in [19])/ca. 100 ♂ ♂ and ♀♀.
 Distribution: AT (BW, NAM, ZW)

Pseudocypris gibbera Sars, 1924

- Barnard [19]: N'kate (=Nekati) Pan, locality no 1510a/approx. coord. 20°05'00" S, 26°01'00" E, 920 m a.s.l./FEOW: 570 Kalahari, Habitat: a pan/Coll. date: 8 August 1930/two empty valves;
- 2. Martens [24]: collected by the Vernay-Lang Kalahari expedition 1930, no further details given (probably same localities as in [19])/few specimens.

Distribution: AT (BW, NAM)

Subfamily: Cypridopsinae Kaufmann, 1900 Tribe: Cypridopsini Kaufmann, 1900 Genus: *Cypridopsis* Brady, 1867

*Cypridopsis vidua (O.F. Müller, 1776)

- Present paper: Kang Nkisi Guest House in the village of Kang, site no SA-95/coord. 23°04′50″ S, 22°45′55″ E, 1142 m a.s.l./FEOW: 570 Kalahari/Habitat: artificial pool/Coll. date: 12 September 2012/7 99, 5 juv;
- Present paper: Botanic Garden in the city of Gabarone, site no SA-112/coord. 24°39′56″ S, 25°56′40″ E, 987 m a.s.l./FEOW: 576 Zambezian Lowveld/Habitat: artificial rockpool/Coll. date: 19 September 2012/11 ♀♀, 28 juv;
- Present paper: Kolobeng river near the village of Manyana, site no SA-113/coord. 24°46′08″ S, 25°35′22″ E, 1133 m a.s.l./FEOW: 576 Zambezian Lowveld/Habitat: river/Coll. date: 20 September 2012/3 φφ, 2 juv;
- Present paper: Ramatlabama river near the village of Ramatlabama, site no SA-117/coord. 25°38′29″ S, 25°34′27″ E, 1276 m a.s.l./FEOW: 571 Southern Kalahari/Habitat: river/Coll. date: 20 September 2012/2 QQ.

Distribution: AT (ZA), AU, NA, NT, OL, PA, PAC

Tribe: Plesiocypridopsini Jacobs & Martens, 2022 Genus: *Plesiocypridopsis* Rome, 1965

Plesiocypridopsis newtoni (Brady & Robertson, 1870)

- McCulloch et al. [21] as *Plesiocypridopsis aldabrae*: North Basin of Makgadikgadi Pan/approx. coord. 20°24′00″ S, 26°12′00″ E, 890 m a.s.l./FEOW: 570 Kalahari/Habitat: ephemeral saline lake (water conductivity 320-24400 µS/cm, pH: 8.6–10.1)/Coll. dates: beginning of the 1999–2000 flood/low abundance;
- Szwarc et al. [22]: floodplains south of Okavango Delta near the city of Maun, site no SA-103/coord: 19°52′12″ S, 23°20′23″ E, 940 m a.s.l./FEOW: 569 Okavango/Habitat: grassy shore of temporary pond/Coll. date: 15 September 2012/3 ♀♀, 2 ♂♂;
- 3. Present paper: Tati river near the town of Francistown, site no SA-108/coord. 21°10′48″ S, 27°30′44″ E, 982 m a.s.l./FEOW: 576 Zambezian Lowveld/Habitat: pools in riverbed/Coll. date: 18 September 2012/3 juv;
- Present paper: Botanic Garden in the city of Gabarone, site no SA-112/coord. 24°39′56″ S, 25°56′40″ E, 987 m a.s.l./FEOW: 576 Zambezian Lowveld/Habitat: artificial rock-pool/Coll. date: 19 September 2012/61 ♀♀and ♂♂, 283 juv.

Distribution: AT (BW, ZA), OL, PA

Genus: Sarscypridopsis McKenzie, 1977

Sarscypridopsis cf. elizabethae (Sars, 1924)

Szwarc et al. [22]: Thamalakane river near the city of Maun, site no SA-97/coord. 19°55′52″ S, 23°30′38″ E, 940 m a.s.l./FEOW: 569 Okavango/Habitat: river side channel/Coll. date: 13 September 2012/19 ♀♀, 1 ♂, 2 juv.

Sarscypridopsis glabrata (Sars, 1924)

Riedel et al. [36]: sediment core 1.5 km east of Kubu Island at the south-western edge of Sua Pan in Makgadikgadi Basin/coord. 20°53'30″ S, 25°50'30″ E, 900–903 m a.s.l./FEOW: 570 Kalahari/Habitat: palaeo-mega-lake system (sediment core to 3.0 m depth)/Coll. dates: September 2007, April 2008, July 2010/fossil valves in a sedimentary sequence dated from ca. 37 ka to 2 ka cal. BP.

Distribution: AT (BW, NAM, ZA)

Sarscypridopsis cf. gregaria Sars, 1895

- Jocqué et al. [20] as Sarscypridopsis cf. gregaria: Kgale Siding near Gabarone/coord. 24°40'30" S, 25°50'20" E, 1040 m a.s.l./FEOW: 576 Zambezian Lowveld/Habitat: nine temporary granite rock pools/Coll. dates: entire inundation cycle of the rainy season of 2002–2003/number of specimens unspecified;
- Jocqué et al. [20,37] as Sarscypridopsis cf. gregaria: near Thamaga/coord. 24°41′50″ S, 25°31′00″ E in [20] and 24°40′30″ S, 25°31′00″ E in [37], 1105 m a.s.l./FEOW: 576 Zambezian Lowveld/Habitat: nine temporary granite rock pools/Coll. dates: entire inundation cycle of the rainy season of 2002–2003/number of specimens unspecified.

Sarscypridopsis harundineti Szwarc et al., 2021

- 1. Szwarc et al. [22]: Lake Ngami, site no SA-96/coord: 20°28′57″S, 22°42′08″E, 930 m a.s.l./FEOW: 569 Okavango/Habitat: endorheic lake/Coll. date: 12 September 2012/1 juv;
- Szwarc et al. [22]: Thamalakane river near the city of Maun, site no SA-97/coord. 19°55′52″ S, 23°30′38″ E, 940 m a.s.l./FEOW: 569 Okavango/Habitat: river side channel/Coll. date: 13 September 2012/11 99, 1 juv;
- Szwarc et al. [22]: floodplains south of Okavango Delta near the city of Maun, site no SA-98/coord: 19°52′15″ S, 23°21′06″ E, 940 m a.s.l/FEOW: 569 Okavango/Habitat: temporary channel/Coll. date: 14 September 2012/6 φφ;
- Szwarc et al. [22]: floodplains south of Okavango Delta near the city of Maun, site no SA-99/coord: 19°52′15″ S, 23°20′45″ E, 940 m a.s.1/FEOW: 569 Okavango/Habitat: temporary channel/Coll. date: 14 September 2012/16 ♀♀, 1 juv;
- Szwarc et al. [22]: floodplains south of Okavango Delta near the city of Maun, site no SA-100/coord: 19°52′04″ S, 23°20′38″ E, 940 m a.s.1/FEOW: 569 Okavango/Habitat: flooded swamp and grassland/Coll. date: 14 September 2012/11 ♀♀;
- 6. Szwarc et al. [22]: floodplains south of Okavango Delta near the city of Maun, site no SA-101/coord: 19°51′39″ S, 23°19′41″ E, 940 m a.s.l/FEOW: 569 Okavango/Habitat: isolated pool in flooded grassland/Coll. date: 15 September 2012/6 ♀♀;
- Szwarc et al. [22]: floodplains south of Okavango Delta near the city of Maun, site no SA-102/coord: 19°52′06″ S, 23°20′41″ E, 940 m a.s.1/FEOW: 569 Okavango/Habitat: floodplain channel/Coll. date: 15 September 2012/1 9;
- Szwarc et al. [22]: North-West District, floodplains south of Okavango Delta near the city of Maun, site no SA-103/coord: 19°52′12″ S, 23°20′23″ E, 940 m a.s.l./FEOW: 569 Okavango/Habitat: grassy shore of temporary pond/Coll. date: 15 September 2012/28 ♀♀.

Distribution: AT (BW)

*Sarscypridopsis cf. reniformis (Sars, 1924)

Present paper: Ramatlabama river near the village of Ramatlabama, site no SA-117/coord. 25°38'29" S, 25°34'27" E, 1276 m a.s.l./FEOW: 571 Southern Kalahari/Habitat: river/Coll. date: 20 September 2012/76 99, 121 juv.

Sarscypridopsis sp.

Jocqué et al. [20]: near Thamaga/coord. 24°41′50″ S, 25°31′00″ E, 1105 m a.s.l./FEOW: 576 Zambezian Lowveld/Habitat: nine temporary granite rock pools/Coll. dates: entire inundation cycle of the rainy season of 2002–2003/number of specimens unspecified.

Tribe: Potamocypridini Ghetti & McKenzie, 1981 Genus: *Potamocypris* Brady, 1870 Potamocypris deflexa (Sars, 1924)

Szwarc et al. [22]: floodplains south of Okavango Delta near the city of Maun, site no SA-99/coord: 19°52'15″ S, 23°20'45″ E, 940 m a.s.l/FEOW: 569 Okavango/Habitat: temporary channel/Coll. date: 14 September 2012/2 99. Distribution: AT (BW, ZA)

Potamocypris mastigophora (Methuen, 1910)

- Barnard [19] as *Cyprilla producta* Sars: Kaotwe Pan, locality no 374/approx. coord. 22°33′00″ S, 23°15′00″ E, 1011 m a.s.l./FEOW: 570 Kalahari/Habitat: a pan/Coll. date: 10 April 1930/very numerous;
- Olszewski et al. [34]: Makgadikgadi depression/coord: 20°08'02" S, 25°33'41" E, 934 m a.s.l./FEOW: 570 Kalahari/Habitat: raised from a sample of dry sediment from temporary shallow salt lake/Coll. date: 19 September 2012/number of specimens unspecified;
- 3. Szwarc et al. [22]: Thamalakane river near the city of Maun, site no SA-97/coord. 19°55′52″ S, 23°30′38″ E, 940 m a.s.l./FEOW: 569 Okavango/Habitat: river side channel/Coll. date: 13 September 2012/697 ♀♀and ♂♂, 135 juv;
- Szwarc et al. [22]: floodplains south of Okavango Delta near the city of Maun, site no SA-99/coord: 19°52′15″ S, 23°20′45″ E, 940 m a.s.l/FEOW: 569 Okavango/Habitat: temporary channel/Coll. date: 14 September 2012/1 ♀;
- Present paper: Mahalapye river near the town of Mahalapye, site no SA-110/coord. 23°06′09″ S, 26°50′21″ E, 1006 m a.s.l./FEOW: 576 Zambezian Lowveld/Habitat: small river dam/Coll. date: 18 September 2012/8 φφ, 11 juv;
- Present paper: Botanic Garden in the city of Gabarone, site no SA-112/coord. 24°39′56″ S, 25°56′40″ E, 987 m a.s.l./FEOW: 576 Zambezian Lowveld/Habitat: artificial rock-pool/Coll. date: 19 September 2012/595 ♀♀and ♂♂, 679 juv;
- Present paper: Bathoen near the town of Kanye, site no SA-114/coord. 24°56′54″ S, 25°20′39″ E, 1255 m a.s.l./FEOW: 575 Southern Temperate Highveld/Habitat: river flood pool/Coll. date: 20 September 2012/1 φ.

Distribution: AT (BW), PA

Potamocypris variegata (Brady & Norman, 1889)

Riedel et al. [36]: sediment core 1.5 km east of Kubu Island at the south-western edge of Sua Pan in Makgadikgadi Basin/coord. 20°53′30″ S, 25°50′30″ E, 900–903 m a.s.l./FEOW: 570 Kalahari/Habitat: palaeo-mega-lake system (sediment core to 3.0 m depth)/Coll. dates: September 2007, April 2008, July 2010/fossil valves in a sedimentary sequence dated from ca. 37 ka to 2 ka cal. BP.

Distribution: AT (BW), NA, PA

Potamocypris sp.

Jocqué et al. [20] and Jocqué et al. [37] as *Potamocypris* spec: near Thamaga/coord. 24°41′50″ S, 25°31′00″ E, 1105 m a.s.l./FEOW: 576 Zambezian Lowveld/Habitat: nine temporary granite rock pools/Coll. dates: entire inundation cycle of the rainy season of 2002–2003/number of specimens unspecified.

Potamocypris new sp. ?

McCulloch et al. [21]: North Basin of Makgadikgadi Pan/approx. coord. $20^{\circ}24'00''$ S, $26^{\circ}12'00''$ E, 905 m a.s.l./FEOW: 570 Kalahari/Habitat: ephemeral saline lake (water conductivity 320-24400 μ S/cm, pH: 8.6–10.1)/Coll. dates: beginning of the 1999–2000 flood/low abundance.

Tribe: Zonocypridini Higuti & Martens, 2012

Genus: Zonocypris G.W. Müller, 1898

Zonocypris costata (Vávra, 1897)

Szwarc et al. [22]: floodplains south of Okavango Delta near the city of Maun, site no SA-99/coord: 19°52'15″ S, 23°20'45″ E, 940 m a.s.l/FEOW: 569 Okavango/Habitat: temporary channel/Coll. date: 14 September 2012/1 ♀. Distribution: AT (BW, ZA), PA

Zonocypris tuberosa G.W. Müller, 1908

Szwarc et al. [22]: floodplains south of Okavango Delta near the city of Maun, site no SA-100/coord: 19°52′04″ S, 23°20′38″ E, 940 m a.s.l/FEOW: 569 Okavango/Habitat: flooded swamp and grassland/Coll. date: 14 September 2012/1 Q.

Distribution: AT (BW, ZA)

Subfamily: Cyprinotinae Bronstein, 1947 Genus: *Hemicypris* Sars, 1903

Hemicypris inversa (Daday, 1913)

 Daday [18] as *Cyprinotus inversus*: Ku-Gudië between Phitshane and Kooa, South Kalahari, site no 5/approx. coord. 25°40′00″ S, 25°06′00″ E, 1100–1200 m a.s.l./FEOW: 571 Southern Kalahari/Habitat: unspecified/Coll. date: January 1905/numerous females and males;

Note: Original locality name Ku-Gudië could not be found. Approximate coordinates are estimated based on the description of the Kalahari Expedition by Schultze [40], where a detailed collection date can also be found as 25 January 1907.

- 2. Smith [41]: specimens from unspecified locality in Botswana originated most probably from the Schultze Kalahari Expedition/duplicate record of [18];
- Szwarc et al. [22]: Lake Ngami, site no SA-96/coord: 20°28'57" S, 22°42'08" E, 930 m a.s.l./FEOW: 569 Okavango/Habitat: endorheic lake/Coll. date: 12 September 2012/2 99. Distribution: AT (BW), PA

Hemicypris reticulata (Klie, 1930)

Jocqué et al. [20] as *Hemicypris reticulata*: not indicated from which of the two studied sites the species was recorded, either Kgale Siding (coord. 24°40′30″ S, 25°50′20″ E) or near Thamaga (coord. 24°41′50″ S, 25°31′00″ E)/FEOW: 576 Zambezian Lowveld/Habitat: temporary granite rock pools/Coll. dates: entire inundation cycle of the rainy season of 2002–2003/number of specimens unspecified.

Distribution: AT (BW, ZA), NT, OL

Genus: Heterocypris Claus, 1892

Heterocypris giesbrechti (G.W. Muller, 1898)

 Savatenalinton & Martens [31] as Heterocypris giesbrechti: Ku-Gudië between Phitshane and Kooa, South Kalahari, site no 5/approx. coord. 25°40′00″ S, 25°06′00″ E, 1100–1200 m a.s.l./FEOW: 571 Southern Kalahari/Habitat: unspecified/Coll. date: January 1905/numerous females and males;

Note: Among individuals identified originally by [18] as Hemicypris inversa, the authors distinguished males belonging to Heterocypris giesbrechti.

- Szwarc et al. [22]: Lake Ngami, site no SA-96/coord: 20°28'57" S, 22°42'08" E, 930 m a.s.l./FEOW: 569 Okavango/Habitat: endorheic lake/Coll. date: 12 September 2012/1 ♀, 3 ♂♂, 7 juv;
- 3. Szwarc et al. [22]: floodplains south of Okavango Delta near the city of Maun, site no SA-98/coord: 19°52′15″ S, 23°21′06″ E, 940 m a.s.l/FEOW: 569 Okavango/Habitat: temporary channel/Coll. date: 14 September 2012/2 ♀♀, 9 juv.

Distribution: AT (BW, ZA), OL

Heterocypris incongruens (Ramdohr, 1808)

Jocqué et al. [37] as *Heterocypris incongruens*: near Thamaga/coord. 24°40'30" S, 25°31'00" E, 1105 m a.s.l./FEOW: 576 Zambezian Lowveld/Habitat: granite rock pools, surfacing in a mainly savannah matrix of grasslands and shrubs/Coll. dates: 15 January–2 March 2003/number of specimens unspecified.

Distribution: AT (BW), AU, NA, NT, OL, PA, PAC

Heterocypris cf. incongruens (Ramdohr, 1808)

Present paper: Ramatlabama river near the village of Ramatlabama, site no SA-117/coord. 25°38'29" S, 25°34'27" E, 1276 m a.s.l./FEOW: 571 Southern Kalahari/Habitat: river/Coll. date: 20 September 2012/8 99, 72 juv.

Heterocypris oblonga (Sars, 1924)

- Szwarc et al. [22]: Thamalakane river near the city of Maun, site no SA-97/coord. 19°55′52″ S, 23°30′38″ E, 940 m a.s.l./FEOW: 569 Okavango/Habitat: river side channel/Coll. date: 13 September 2012/132 ♀♀and ♂♂, 90 juv;
- Szwarc et al. [22]: floodplains south of Okavango Delta near the city of Maun, site no SA-99/coord: 19°52'15" S, 23°20'45" E, 940 m a.s.l/FEOW: 569 Okavango/Habitat: temporary channel/Coll. date: 14 September 2012/153 99, 106 juv;
- 3. Szwarc et al. [22]: floodplains south of Okavango Delta near the city of Maun, site no SA-100/coord: 19°52′04″ S, 23°20′38″ E, 940 m a.s.1/FEOW: 569 Okavango/Habitat: flooded swamp and grassland/Coll. date: 14 September 2012/14 ♀♀, 6 juv;
- 4. Szwarc et al. [22]: floodplains south of Okavango Delta near the city of Maun, site no SA-101/coord: 19°51′39″ S, 23°19′41″ E, 940 m a.s.1/FEOW: 569 Okavango/Habitat: isolated pool in flooded grassland/Coll. date: 15 September 2012/25 ♀♀, 17 ♂♂, 1 juv;
- Szwarc et al. [22]: floodplains south of Okavango Delta near the city of Maun, site no SA-102/coord: 19°52′06″ S, 23°20′41″ E, 940 m a.s.l/FEOW: 569 Okavango/Habitat: floodplain channel/Coll. date: 15 September 2012/1 Q, 2 juv;
- Szwarc et al. [22]: North-West District, floodplains south of Okavango Delta near the city of Maun, site no SA-103/coord: 19°52'12" S, 23°20'23" E, 940 m a.s.l./FEOW: 569 Okavango/Habitat: grassy shore of temporal pond/Coll. date: 15 September 2012/16 φφ, 2 juv.

Distribution: AT (BW, NAM, ZA)

Heterocypris ovularis (Sars, 1924)

- Barnard [19] as *Herpetocypris ovularis* Sars: 2 miles north of Tsotsoroga, localities nos 1204b/approx. coord. 18°40′00″ S, 24°19′00″ E, 1058 m a.s.l./FEOW: 569 Okavango/Habitat: not specified/Coll. dates: 19 June 1930/mostly empty valves;
- Barnard [19] as *Herpetocypris ovularis* Sars: Kaotwe Pan, locality no 374a/approx. coord. 22°33'00" S, 23°15'00" E, 1011 m a.s.l./FEOW: 570 Kalahari/Habitat: a pan/Coll. date: 10 April 1930/a few specimens. Distribution: AT (BW, NAM, ZA)

Heterocypris sp.

- Jocqué et al. [20]: Kgale Siding near Gabarone/coord. 24°40'30" S, 25°50'20" E, 1040 m a.s.l./FEOW: 576 Zambezian Lowveld/Habitat: nine temporary granite rock pools/Coll. dates: entire inundation cycle of the rainy season of 2002–2003/number of specimens unspecified;
- Jocqué et al. [20] and Jocqué et al. [37] as *Heterocypris* sp. nov.: near Thamaga/coord. 24°41′50″ S, 25°31′00″ E, 1105 m a.s.l./FEOW: 576 Zambezian Lowveld/Habitat: nine temporary granite rock pools/Coll. dates: entire inundation cycle of the rainy season of 2002–2003/number of specimens unspecified.

Subfamily: Herpetocypridinae Kaufmann, 1900 Tribe: Isocypridini Rome, 1965 Genus: *Amphibolocypris* Rome, 1965

Amphibolocypris arida Jocqué & Martens, 2010

- 1. Jocqué et al. [20] as *Amphibolocypris* sp. n. sp.: Kgale Siding near Gabarone/coord. 24°40′30″ S, 25°50′20″ E, 1040 m a.s.l./FEOW: 576 Zambezian Lowveld/Habitat: nine temporary granite rock pools/Coll. dates: entire inundation cycle of the rainy season of 2002–2003/number of specimens unspecified;
- Jocqué et al. [20] as Amphibolocypris sp. n. sp.: near Thamaga/coord. 24°41′50″ S, 25°31′00″ E, 1105 m a.s.l./FEOW: 576 Zambezian Lowveld/Habitat: nine temporary granite rock pools/Coll. dates: entire inundation cycle of the rainy season of 2002– 2003/number of specimens unspecified;
- Jocqué et al. [37]: near Thamaga/coord. 24°40′30″ S, 25°31′00″ E, 1105 m a.s.l./FEOW: 576 Zambezian Lowveld/Habitat: granite rock pools in a mainly savannah matrix of grasslands and shrubs/Coll. dates: 15 January–2 March 2003/type material consisted of 3 ♀♀and 3 ♂♂ (duplicate record of [20]).

Distribution: AT (BW)

Genus: Isocypris G.W. Müller, 1908

Isocypris cf. priomena G.W. Müller, 1908

- Szwarc et al. [22]: Thamalakane river near the city of Maun, site no SA-97/coord. 19°55′52″ S, 23°30′38″ E, 940 m a.s.l./FEOW: 569 Okavango/Habitat: river side channel/Coll. date: 13 September 2012/2 99, 1 juv;
- Present paper: Bathoen reservoir near the town of Kanye, site no SA-115/coord. 24°57′06″ S, 25°20′33″ E, 1267 m a.s.l./FEOW: 575 Southern Temperate Highveld/Habitat: reservoir/Coll. date: 20 September 2012/50 ♀♀, 353 juv.

Tribe: Stenocypridini Ferguson, 1964 Genus: *Chrissia* Hartmann, 1957

Chrissia fascigera (Sars, 1924)

Barnard [19] as *Stenocypris fascigera* Sars: 1 mile north-east of Tsotsoroga, locality no 1223/approx. coord. 18°42′00″ S, 24°22′00″ E, 930 m a.s.l./FEOW: 569 Okavango/Habitat: not specified/Coll. date: 22 June 1930/number of specimens unspecified.

Distribution: AT (BW, NAM)

*Chrissia cf. pectinata (Sars, 1924)

Present paper: Bathoen near the town of Kanye, site no SA-114/coord. $24^{\circ}56'54''$ S, $25^{\circ}20'39''$ E, 1255 m a.s.l./FEOW: 575 Southern Temperate Highveld/Habitat: river flood pool/Coll. date: 20 September 2012/1 \circ .

Chrissia cf. perarmata (Brady, 1904)

Szwarc et al. [22]: Thamalakane river near the city of Maun, site no SA-97/coord. 19°55′52″ S, 23°30′38″ E, 940 m a.s.l./FEOW: 569 Okavango/Habitat: river side channel/Coll. date: 13 September 2012/1 Q.

Genus: Stenocypris Sars, 1889

Stenocypris malayica Victor and Fernando, 1981

- Szwarc et al. [22]: Thamalakane river near the city of Maun, site no SA-97/coord. 19°55′52″ S, 23°30′38″ E, 940 m a.s.l./FEOW: 569 Okavango/Habitat: river side channel/Coll. date: 13 September 2012/2 ♀♀, 3 juv;
- Szwarc et al. [22]: floodplains south of Okavango Delta near the city of Maun, site no SA-100/coord: 19°52′04″ S, 23°20′38″ E, 940 m a.s.1/FEOW: 569 Okavango/Habitat: flooded swamp and grassland/Coll. date: 14 September 2012/1 ♀.

Distribution: AT (BW), NT, OL, PA, PAC

Subfamily: Megalocypridinae Rome, 1965 Tribe: Megalocypridini Rome, 1965 Genus: *Apatelecypris* Rome, 1965

Apatelecypris schultzei (Daday, 1913)

Barnard [19] as *Megalocypris brevis* Sars: N'kate (= Nekati) Pan, locality no 1504/approx. coord. 20°05'00'' S, 26°01'00'' E, 920 m a.s.l./FEOW: 570 Kalahari/Habitat: a shallow pan in limestone formation/Coll. date: 7 August 1930/number of specimens unspecified. Distribution: AT (BW, NAM)

Genus: Sclerocypris Sars, 1924

Sclerocypris clavularis Sars, 1924

Jocqué et al. [20] as *Sclerocypris clavularis*: not indicated from which of the two studied sites the species was recorded, either Kgale Siding (coord. 24°40′30″ S, 25°50′20″ E) or near Thamaga (coord. 24°41′50″ S, 25°31′00″ E)/FEOW: 576 Zambezian Lowveld/Habitat: temporary granite rock pools/Coll. dates: entire inundation cycle of the rainy season of 2002–2003/number of specimens unspecified.

Distribution: AT (BW, ZA)

Sclerocypris exserta makarikarensis Martens, 1988

- Martens [32] and Seaman et al. [33] as *Sclerocypris excerta* Sars (typographical error of *exserta*): Makgadikgadi Pan/approx. coord. 20°42′00″ S, 24°57′00″ E, 905 m a.s.l./FEOW: 570 Kalahari/Habitat: huge, temporary salt pan endorheic system/Coll. date: 4 May 1957, collected by Rhodesian Schools Exploration Society/number of specimens unspecified;
- McCulloch et al. [21]: North Basin of Makgadikgadi Pan/approx. coord. 20°24'00" S, 26°12'00" E, 905 m a.s.l./FEOW: 570 Kalahari/Habitat: ephemeral saline lake system (water conductivity 320-24400 μS/cm, pH: 8.6–10.1)/Coll. dates: December 1999–June 2001/number of specimens unspecified;
- McCulloch et al. [21]: Middle Basin of Makgadikgadi Pan/approx. coord. 20°39'00" S, 26°04'00" E, 905 m a.s.l./FEOW: 570 Kalahari/Habitat: ephemeral saline lake system (water conductivity 730-91600 μS/cm, pH: 8.6–10.1)/Coll. dates: December 1999–June 2001/ number of specimens unspecified.

Distribution: AT (BW)

Sclerocypris methueni (Kempf, 2015)

Olszewski et al. [34] as *Sclerocypris tuberculata* (Sars) (= *Sclerocypris sarsi* Martens): Makgadikgadi depression/coord: 20°08′02″ S, 25°33′41″ E, 934 m a.s.l./FEOW: 570 Kalahari/Habitat: raised from a sample of dry sediment from temporary shallow salt lake/Coll. date: 19 September 2012/number of specimens unspecified.

Distribution: AT (BW, ZA)

Sclerocypris sp.

Szwarc et al. [22]: Thamalakane river near the city of Maun, site no SA-97/coord. 19°55′52″ S, 23°30′38″ E, 940 m a.s.l./FEOW: 569 Okavango/Habitat: river side channel/Coll. date: 13 September 2012/1 ♂.

Family: Candonidae Kaufmann, 1900 Subfamily: Candoninae Kaufmann, 1900 Tribe: Candonini Kaufmann, 1900 Genus: *Pseudocandona* Kaufmann, 1900

*Pseudocandona sp.

- Present paper: Shashe river near the village of Shashe, site no SA-109/coord. 21°23'20" S, 27°27'20" E, 956 m a.s.l./FEOW: 576 Zambezian Lowveld/Habitat: pools in riverbed/ Coll. date: 18 September 2012/97 99;
- Present paper: Mahalapye river near the town of Mahalapye, site no SA-110/coord. 23°06′09″ S, 26°50′21″ E, 1006 m a.s.l./FEOW: 576 Zambezian Lowveld/Habitat: small river dam/Coll. date: 18 September 2012/1 9;
- Present paper: Bathoen near the town of Kanye, site no SA-114/coord. 24°56′54″ S, 25°20′39″ E, 1255 m a.s.l./FEOW: 575 Southern Temperate Highveld/Habitat: river flood pool/Coll. date: 20 September 2012/10 φφ.

Tribe: Candonopsini Karanovic, 2004 Genus: Candonopsis (Candonopsis) Vávra, 1891

*Candonopsis nama Daday, 1913

Present paper: Bathoen near the town of Kanye, site no SA-114/coord. 24°56′54″ S, 25°20′39″ E, 1255 m a.s.l./FEOW: 575 Southern Temperate Highveld/Habitat: river flood pool/Coll. date: 20 September 2012/20 99, 22 ♂♂, 1 juv.

Distribution: AT (NAM)

Candonopsis navicula Daday, 1910

Szwarc et al. [22]: Thamalakane river near the city of Maun, site no SA-97/coord. $19^{\circ}55'52''$ S, $23^{\circ}30'38''$ E, 940 m a.s.l./FEOW: 569 Okavango/Habitat: river side channel/Coll. date: 13 September 2012/1 \circ .

Distribution: AT (BW) Subfamily: Cyclocypridinae Kaufmann, 1900 Genus: *Physocypria* Vávra, 1897 *Physocypria* cf. *capensis* (Sars, 1895)

- Szwarc et al. [22]: Thamalakane river near the city of Maun, site no SA-97/coord. 19°55′52″ S, 23°30′38″ E, 940 m a.s.l./FEOW: 569 Okavango/Habitat: river side channel/Coll. date: 13 September 2012/1 ♀;
- Present paper: Kolobeng river near the village of Manyana, site no SA-113/coord. 24°46′08″ S, 25°35′22″ E, 1133 m a.s.l./FEOW: 576 Zambezian Lowveld/Habitat: river/Coll. date: 20 September 2012/15 ♀♀, 46 juv;
- 3. Present paper: Bathoen near the town of Kanye, site no SA-114/coord. 24°56′54″ S, 25°20′39″ E, 1255 m a.s.l./FEOW: 575 Southern Temperate Highveld/Habitat: river flood pool/Coll. date: 20 September 2012/5 ♀♀, 3 juv.

Family: Ilyocyprididae Kaufmann, 1900 Subfamily: Ilyocypridinae Kaufmann, 1900 Genus: *Ilyocypris* Brady & Norman, 1889 *Ilyocypris cf. gibba (Ramdohr, 1808)

- 1. Present paper: Tati river near the town of Francistown, site no SA-108/coord. 21°10'48" S, 27°30′44″ E, 982 m a.s.l./FEOW: 576 Zambezian Lowveld/Habitat: pools in riverbed/ Coll. date: 18 September $2012/2 \circ \circ;$
- 2. Present paper: Mahalapye river near the town of Mahalapye, site no SA-110/coord. 23°06′09″ S, 26°50′21″ E, 1006 m a.s.l./FEOW: 576 Zambezian Lowveld/Habitat: small river dam/Coll. date: 18 September 2012/8 99, 2 juv;
- Present paper: Bathoen near the town of Kanye, site no SA-114/coord. 24°56′54″ S, 3. 25°20'39" E, 1255 m a.s.l./FEOW: 575 Southern Temperate Highveld/Habitat: river flood pool/Coll. date: 20 September 2012/1 9, 1 juv.

Ilyocypris sp.

- 1. Riedel et al. [35]: an outcrop on Kubu Island at the south-western edge of Sua Pan in Makgadikgadi Basin/coord. 20°53′29″ S, 25°51′14″ E, 901 m a.s.l./FEOW: 570 Kalahari/Habitat: palaeo-mega-lake system (sediments representing relics of the 8.5 ka B.P. last "mega-lake event")/Coll. dates: September 2007, April 2008, July 2010/fossil valves;
- 2. Riedel et al. [36]: sediment core 1.5 km east of Kubu Island at the south-western edge of Sua Pan in Makgadikgadi Basin/coord. 20°53′30″ S, 25°50′30″ E, 900–903 m a.s.l./FEOW: 570 Kalahari/Habitat: palaeo-mega-lake system (core to 3.0 m depth)/ Coll. dates: September 2007, April 2008, July 2010/fossil valves in a sedimentary sequence dated from ca. 37 ka to 2 ka cal. BP.

**Ilyocypris* sp. n.

Present paper: Mahalapye river near the town of Mahalapye, site no SA-110/coord. 23°06'09" S, 26°50'21" E, 1006 m a.s.l./FEOW: 576 Zambezian Lowveld/Habitat: small river dam/Coll. date: 18 September 2012/a few empty valves.

Note: A species resembling representatives of the fossil genus Juxilyocypris Kempf, 2011 known exclusively from Pleistocene to Holocene deposits in Europe ([42,43]).

Family: Notodromadidae Kaufmann, 1900 Subfamily: Oncocypridinae De Deckker, 1979 Genus: Oncocypris G.W. Müller, 1898

*Oncocypris muelleri (Daday, 1910)

- 1. Present paper: Bathoen reservoir near the town of Kanye, site no SA-115/coord. 24°57'06" S, 25°20'33" E, 1267 m a.s.l./FEOW: 575 Southern Temperate Highveld/Habitat: reservoir/Coll. date: 20 September 2012/subfossil empty valves;
- 2. Present paper: Moshenang Dam near the village of Kanye, site no SA-116/coord. 24°54′50″ S, 25°16′15″ E, 1282 m a.s.l./FEOW: 576 Zambezian Lowveld/Habitat: reservoir/Coll. date: 20 September 2012/subfossil empty valves.

Distribution: AT (ZA)

Superfamily: Cytheroidea Baird, 1850 Family: Limnocytheridae Sars, 1925 Subfamily: Limnocytherinae Sars, 1925 Tribe: Limnocytherini Klie, 1938 Genus: Limnocythere Brady, 1867

Limnocythere inopinata (Baird, 1843)

Riedel et al. [36]: sediment core 1.5 km east of Kubu Island at the south-western edge of Sua Pan in Makgadikgadi Basin/coord. 20°53'30" S, 25°50'30" E, 900–903 m a.s.l./FEOW: 570 Kalahari/Habitat: palaeo-mega-lake system (core to 3.0 m depth)/Coll. dates: September 2007, April 2008, July 2010/fossil valves in a sedimentary sequence dated from ca. 37 ka to 2 ka cal. BP.

Distribution: AT (BW, ZA), NA, PA

Limnocythere cf. stationis Vavra, 1891

- Szwarc et al. [22]: Thamalakane river near the city of Maun, site no SA-97/coord. 19°55′52″ S, 23°30′38″ E, 940 m a.s.l./FEOW: 569 Okavango/Habitat: river side channel/Coll. date: 13 September 2012/8 ♀♀, 2 juv;
- Szwarc et al. [22]: North-West District, floodplains south of Okavango Delta near the city of Maun, site no SA-103/coord: 19°52′12″ S, 23°20′23″ E, 940 m a.s.l./FEOW: 569 Okavango/Habitat: grassy shore of temporary pond/Coll. date: 15 September 2012/1 9;
- Present paper: Shashe river near the village of Shashe, site no SA-109/coord. 21°23′20″ S, 27°27′20″ E, 956 m a.s.l./FEOW: 576 Zambezian Lowveld/Habitat: pools in riverbed/ Coll. date: 18 September 2012/10 99, 1 juv;
- Present paper: Mahalapye river near the town of Mahalapye, site no SA-110/coord. 23°06′09″ S, 26°50′21″ E, 1006 m a.s.l./FEOW: 576 Zambezian Lowveld/Habitat: small river dam/Coll. date: 18 September 2012/3 φφ;
- Present paper: Bathoen reservoir near the town of Kanye, site no SA-115/coord. 24°57′06″ S, 25°20′33″ E, 1267 m a.s.l./FEOW: 575 Southern Temperate Highveld/ Habitat: reservoir/Coll. date: 20 September 2012/28 §§.

Limnocythere thomasi Martens, 1990

- Riedel et al. [35] as *Limnocythere thomasi*–group: an outcrop on Kubu Island at the south-western edge of Sua Pan in Makgadikgadi Basin/coord. 20°53'309" S, 25°49'00" E, 908 m a.s.l./FEOW: 570 Kalahari/Habitat: palaeo-mega-lake system (sediments representing relics of the 8.5 ka B.P. last "mega-lake event")/Coll. dates: September 2007, April 2008, July 2010/fossil valves;
- Riedel et al. [36]: sediment core 1.5 km east of Kubu Island at the south-western edge of Sua Pan in Makgadikgadi Basin/coord. 20°53'30" S, 25°50'30" E, 900–903 m a.s.l./FEOW: 570 Kalahari/Habitat: palaeo-mega-lake system (core to 3.0 m depth)/ Coll. dates: September 2007, April 2008, July 2010/fossil valves in a sedimentary sequence dated from ca. 37 ka to 2 ka cal. BP.

Distribution: AT (BW)

Limnocythere tudoranceai Martens, 1990

- 1. Martens et al. [44]: no further details given (not listed from Botswana also in the checklist of Martens [17]);
- McCulloch et al. [21]: North Basin of Makgadikgadi Pan/approx. coord. 20°25′00″ S, 26°11′00″ E, 890 m a.s.l./FEOW: 570 Kalahari/Habitat: temporary salt pan/Coll. dates: December 1999–June 2001/number of specimens unspecified;
- McCulloch et al. [21]: Middle Basin of Makgadikgadi Pan/approx. coord. 20°41′00″ S, 26°06′00″ E, 904 m a.s.l./FEOW: 570 Kalahari/Habitat: temporary salt pan/Coll. dates: December 1999–June 2001/number of specimens unspecified;
- McCulloch et al. [21]: South Basin of Makgadikgadi Pan/approx. coord. 21°00′00″ S, 26°12′00″ E, 904 m a.s.l./FEOW: 570 Kalahari/Habitat: temporary salt pan/Coll. dates: December 1999–June 2001/number of specimens unspecified.

Distribution: AT (BW, NAM)

Limnocythere sp.

Present paper: Nata river near the village of Nata, site no SA-107/coord. 20°12′55″ S, 26°11′09″ E, 915 m a.s.l./FEOW: 570 Kalahari/Habitat: pools in riverbed/Coll. date: 17 September 2012/ empty valves.

The inventory of living and (sub)fossil non-marine Ostracoda of Botswana is presented in Table 1.

Table 1. Checklist of living (L), subfossil (SF = empty valves found in samples of wet surface sediment of existing waterbodies), and fossil (F = valves from Late-Pleistocene-Holocene sediments) non-marine Ostracoda from Botswana. Records of our own, unpublished field collection are denoted by X in the Reference column, and species with first records for Botswana are marked with an asterisk (*). Records included in the previous checklist of Ostracoda of the region of Southern Africa [16] are indicated in the brackets ([16]) after the original references.

No.	Species	References	Fossil/Living	No.	Species	References	Fossil/Living
1.	Amphibolocypris arida	[20,37]	L	28.	*Oncocypris muelleri	Х	SF
2.	Apatelecypris schultzei	[19] ([16])	L	29.	Physocypria cf. capensis	[22], X	L
3.	*Candonopsis nama	Х	L	30.	Plesiocypridopsis newtoni	[21,22], X	L
4.	Candonopsis navicula	[22]	L	31.	Potamocypris deflexa	[22]	L
5.	Chrissia fascigera	[19] ([16])	L	32.	Potamocypris mastigophora	[19,22,34], X ([16])	L
6.	*Chrissia cf. pectinata	Х	L	33.	Potamocypris variegata	[36]	F
7.	Chrissia cf. perarmata	[22]	L	34.	Potamocypris new sp. ?	[21]	L
8.	*Cypricercus cf. cuneatus	Х	L	35.	Potamocypris sp.	[20,37]	L
9.	Cypricercus inermis	[20]	L	36.	*Pseudocandona sp.	Х	L
10.	*Cypridopsis vidua	Х	L	37.	Pseudocypris circularis	[16,19,24] ([16])	L
11.	Hemicypris inversa	[18,22,41] ([16])	L	38.	Pseudocypris gibbera	[19,24] ([16])	L
12.	Hemicypris reticulata	[20]	L	39.	Sarscypridopsis cf. elizabethae	[22]	L
13.	Heterocypris giesbrechti	[22,31]	L	40.	Sarscypridopsis glabrata	[36]	F
14.	Heterocypris incongruens	[37]	L	41.	Sarscypridopsis cf. gregaria	[20,37]	L
15.	Heterocypris cf. incongruens	Х	L	42.	Sarscypridopsis harundineti	[22]	L
16.	Heterocypris oblonga	[22]	L	43.	*Sarscypridopsis cf. reniformis	Х	L
17.	Heterocypris ovularis	[19] ([16])	L	44.	Sarscypridopsis sp.	[20]	L
18.	Heterocypris sp.	[20,37]	L	45.	Sclerocypris clavularis	[20]	L
19.	*Ilyocypris cf. gibba	Х	L	46.	Sclerocypris exserta makarikarensis	[21,32,33]	L
20.	Ilyocypris sp.	[35,36]	F	47.	Sclerocypris methueni	[34]	L
21.	* <i>Ilyocypris</i> sp. n.	Х	SF	48.	Sclerocypris sp.	[22]	L
22.	Isocypris cf. priomena	[22], X	L	49.	Stenocypris malayica	[22]	L
23.	Limnocythere inopinata	[36]	F	50.	Strandesia cf. prava	[22], X	L
24.	Limnocythere cf. stationis	[22], X	L	51.	<i>Strandesia</i> n. sp. (gr. <i>sudanica</i>)	[20]	L
25.	Limnocythere thomasi	[35,36]	F	52.	<i>Strandesia</i> sp.	[36]	F
26.	Limnocythere tudoranceai	[21,44]	L	53.	Zonocypris costata	[22]	L
27.	Limnocythere sp.	Х	SF	54.	Zonocypris tuberosa	[22]	L

3.2. Biodiversity of Non-Marine Ostracods of Botswana

Although numbers of specimens were recorded in our material, the results presented here have a qualitative character, and species incidence was investigated rather than abundance to be comparable with the published results of other studies. Our survey produced 17 records of ostracod species from 11 new locations, mainly from the east and south-east of Botswana (Figure 1, Table 1). Together with other published records, the present checklist includes, in total, 54 (45 living and nine fossil or subfossil) ostracod species currently reported from Botswana, with 23 taxa left in open nomenclature. Of the total number, nine species were found only as empty valves either in samples of wet surface sediment of the existent waterbodies (four taxa of subfossil material) or in the Late-Pleistocene-Holocene sediments (Table 1). The majority (75.9%) of the species in the checklist belong to the family Cyprididae.

The accumulation plot of the observed species number was unsaturated, not reaching asymptotic levelling-off (Figure 2). The total observed species richness, based on living ostracod records from 28 sites (11 own sites, eight from [22], three from [21], three from [19] supplemented by [24], two from [20,37], and one from [18] revised by [31]) was 65.2% of the species number estimated by the Chao 2 index (mean \pm SD = 69.0 \pm 13.31).

Compared with the total (gamma) diversity (54 species of the total inventory), alpha diversity expressed by species richness at the individual sampling site was low and ranged from 1 to 12 (median = 3, mean \pm SD = 3.3 \pm 2.25), with four sites having only one species recorded. The most common species based on the samples from the above-



mentioned 28 sites were *Sarscypridopsis harundineti* (eight sites, 28.6% of the total 28) as well as *Heterocypris oblonga* and *Potamocypris mastigophora* (both present at six sites, 21.4%).

Figure 2. Species accumulation curves showing the average observed (closed circles) and cumulative taxa richness estimated by the Chao 2 index (open circles) against the number of 28 sites of living material. Whiskers display $1 \times SD$ (standard deviation).

Species were mainly distributed across the freshwater ecoregions of Okavango, Zambezian Lowveld, and Kalahari (22, 21, and 18, respectively), where the highest number of sites were sampled. Two other freshwater ecoregions (Southern Kalahari and Southern Temperate Highveld) were represented by only two sampling sites each, and were thus not included in the statistical tests. The ANOSIM showed significant differences among the three mentioned most frequently sampled freshwater ecoregions (Global R = 0.314, p < 0.001). Pairwise tests showed significant differences for Okavango vs. Zambezian Lowveld (R = 0.339, p = 0.001) and Okavango vs. Kalahari (R = 0.488, p < 0.001). The main species principally responsible for this separation included *Sarscypridopsis harundineti* and *Heterocypris oblonga*. The pattern exhibited by the ecoregions on the basis of their ostracod fauna is presented by a non-metric, Multi-Dimensional Scaling (mMDS) plot on bootstrap averages (Figure 3).

Similar results were obtained when the homogeneity of multivariate dispersions (PER-MDIP) test was used to assess differences in beta diversity among the three most-intensively sampled freshwater ecoregions (Okavango, Zambezian Lowveld, and Kalahari). PER-MDISP showed significant differences among these ecoregions (F = 8.869, *p* (perm) = 0.008), and pairwise tests showed significant differences for Okavango vs. Zambezian Lowveld (t = 4.042, *p* = 0.002) and Okavango vs. Kalahari (t = 3.592, *p* = 0.006).



Figure 3. Metric multi-dimensional scaling ordination of ostracod samples with group averages (black symbols) and approximate 95% region estimates fitted to bootstrap averages (shaded areas) for three freshwater ecoregions: Okavango (triangles), Kalahari (squares), and Zambezian Lowveld (circles).

4. Discussion

The present field collection with the collation of previously and recently published surveys brings the total number of living, subfossil, and Late-Pleistocene-Holocene species of non-marine Ostracoda of Botswana to 54 (Table 1) and places this country, together with South Africa (122 species according to Martens [17]) and Namibia (52 species according to Curtis [45] and Curtis et al. [46]), in the group of South African countries having the highest species record. The highest ostracod species richness in South Africa may partly be explained by the largest surface and greatest topographic, climatic, and environmental diversity of this country in the region. A detailed discussion of the distribution and zoogeography of ostracods of this region is very unevenly distributed and vast areas still remain to be investigated. Only limited surveys have been done in Angola, Mozambique, Zambia, and Zimbabwe, which produced only a few records of non-marine ostracods from each of these countries (see [17,47]).

Out of 54 species (including nine fossil or subfossil records) reported so far from Botswana, 23 are unfortunately left in open nomenclature due to the immature taxonomy of Southern African ostracods. These records need thorough taxonomic verification and several of them cannot be considered new species until more morphological (and molecular) evidence is available. At least some of these records, however, are new taxa just awaiting formal descriptions (see e.g., *Strandesia* n. sp. in [20] or *Ilyocypris* sp. n. and *Pseudocandona* sp. in the present paper). This indicates the urgent need for sound systematic studies on harmonizing taxonomy of Southern African ostracods. Nevertheless, of 17 species yielded by our own survey of 11 sites, nine are new records for Botswana (Table 1).

The observed and estimated species richness may suggest that the sampling effort is still not perfectly adequate to represent the number of ostracod species in Botswana. The recorded species amounted to 65% of the estimated species richness (Figure 2). While further sampling may increase the range of some of already reported taxa, the continuing increase in species number recorded from the country over time and non-saturated species accumulation curve clearly indicate that additional range-restricted species are likely to be found, and, thus, support the assertion that the recorded ostracod species richness in Botswana is still considerably underestimated. Most previous sampling sites were situated in the northern part of the country, while our survey extends the ostracod distribution records in the south and east of Botswana (Figure 1). Yet, significant regions of the country still have not been investigated, especially the most arid south-west region with difficult accessibility. No significant efforts have also been made to investigate ostracods in the permanent marshlands and seasonally flooded plains of the vast Okavango Delta system, although ostracods, as a group, have been reported frequently from this area (e.g., [48–50]). The general level of taxonomic exploration of hydrobionts in the Okavango freshwater ecoregion is considered reasonable and the delineation of the ecoregion justified [14,51]. Showing significant differences in ostracod species composition (with endemic Sarscypridopsis harundineti [22]) and lower beta diversity of the Okavango ecoregion versus other two ecoregions of Botswana (Kalahari and Zambezian Lowveld), our preliminary results seem to support the distinctiveness of the Okavango ecoregion. The microcrustacean (mainly planktonic cladocerans and copepods) and Odonata faunas of the Okavango Delta have close affinities with their counterparts of the Bangweulu swamps in Zambia [51,52], while other groups of Okavango aquatic invertebrates biogeographically resemble those occurring in the catchments of the Zambezi and Congo Rivers [14]. Additional larger-scale studies of subcontinental range are needed to investigate the biogeographical relationships between various catchments to locate evidence for former connections and geomorphological evolution of the river systems in Southern Africa. There is also a need for ecological studies, e.g., on salinity tolerances or effects of increasing temperature; thus, any taxonomic and faunistic surveys must routinely be accompanied by records of water and sediment properties. In future research, high priority should also be given to yet unexplored areas and habitats, e.g., there remains much to be learned about the ostracod distribution in the groundwater of various aquifers in Botswana.

The taxonomic composition of the ostracod fauna of Botswana shows that representatives of the family Cyprididae make up 76% of the total specific diversity of the country. This is expected in an arid land such as Botswana, where most of waterbodies are temporary, as species of this family can produce drought-resistant eggs, which can survive unfavorable conditions and can be passively dispersed via zoochory or anemochory to other waterbodies [15,17]. The most species-rich subfamily of Cyprididae in the ostracod fauna of Botswana was Cypridopsinae (24% of the total species inventory), two species of which (*Sarscypridopsis harundineti* and *Potamocypris mastigophora*) were also the most common at the sites considered in the present checklist (eight and six sites of the total of 28, respectively). The Cypridopsinae is also one of the most common and most taxonomically difficult subfamilies of non-marine ostracods in Southern Africa [17].

Although we encountered some species in the ostracod fauna of Botswana that are widespread geographically, e.g., cosmopolitan (Cypridopsis vidua and Heterocypris incongruens) or nearly cosmopolitan (*Ilyocypris gibba* and *Stenocypris malayica*), the majority (23 species, approx. 43%) are restricted to the Afrotropical region or originally recorded and described from that region and subsequently also reported elsewhere in one or two other zoogeographical regions (seven species, 13%). There are relatively few known endemics for Botswana: Amphibolocypris arida from rock pools in the south of the country within the ecoregion of Zambazian Lowveld [20,27], Sarscypridopsis harundineti from floodplains south of Okavango Delta near the city of Maun [22], and *Sclerocypris exserta makarikarensis* from the Makgadikgadi ephemeral saline lake system within the Kalahari ecoregion [21,32]. Even if a cautiously possible four more species (undescribed new species left in open nomenclature) are added to the list of species endemic to Botswana, the overall level of endemism is still low compared with Namibia, where 18 species of the total of 52 (i.e., ca. 35%) are considered endemics [45,46] or with the Western and Eastern Cape provinces of South Africa with several endemic species [10,17]. Since our present knowledge of the ostracod distribution in Botswana is limited, and we do not know which species are likely to be endangered, potential endemics are certainly worthy of protection.

Local momentary ostracod species richness (alpha diversity) at the individual sampling sites included in the present ostracod checklist of Botswana was rather low and ranged from 1 to 12 (mean = 3.3). In a study on ostracods from 60 permanent and temporary waterbodies in the Eastern Cape province of South Africa, Martens and de Moor [53] reported a similar range and mean value of alpha diversity (1–11; mean ca. 4.7). The study of Martens et al. [44] of ostracods in a shallow, semi-estuarine coastal lake-river-swamp system in the Western Cape province showed alpha diversity varying in a saline gradient between 1 and 6 (with a mean value of 4.4). Considerably lower momentary alpha diversities of other microcrustaceans were found by West and van As [54] in a temporary system of the Nata River in north-eastern Botswana, i.e., 0–1 species of Cladocera and 0–2 species of Copepoda. Obviously, numerous and intrinsically related factors influence biodiversity of temporary inland waters in drier climates, e.g., size, isolation, habitat heterogeneity, longevity and frequency of the inundation period, salinity, pH and ionic composition, oxygen concentration as well as stochastic events, human interventions, and biological interactions [55–57]. Moreover, invertebrate communities of a local system of temporary waterbodies during the course of the inundation period undergo ecological succession ensuing hydrological changes, which finally leads to seasonal, annual, and interannual variations in the species composition, so that (coupled with an often-patchy distribution) at any sampling moment the community represents only a portion of the entire species richness of the community [54,58]. Frequent and severe disturbances may also lead to intense selection and species sorting, resulting in a reduction of the total number of species to most tolerant ones [59]. In that case, sampling success varies with the seasonal conditions, and to gather more complete taxa lists either repeated samples of active communities are required (which is often limited in arid regions with erratic rainfall) or instead studying morphologically banks of drought-resistant eggs deposited in the sediment to reconstruct diversity of dormant communities as proposed by Meyer-Milne et al. [58] for branchiopods in dryland wetlands of South Africa. Such an approach has, however, not yet been explored for ostracods and we do not know if egg morphology is species-specific. Alternatively, sediment egg banks may be studied through environmental DNA analysis as was successfully documented for ostracod identification in surface sediment samples collected from Lake Nam Co on the Tibetan Plateau [60].

Threats to biodiversity of aquatic ecosystems are often more severe when compared to terrestrial ecosystems. In the face of global climate change and increasing anthropogenic pressures on temporary wetlands, investigations of spatial and temporal trends in biodiversity loss, especially in drier climates, are becoming increasingly important [2,3,56,61]. Although an arid land, Botswana has diverse wetland habitats which house a variety of aquatic fauna [61], including at least 50 species of Ostracoda, most of which are specialist species adapted to temporary water conditions. Such species produce dormant eggs that are extremely resistant and withstand recurrent dry periods [62], have some risk-spreading strategies (hatching in fractions during a single inundation [63]), and disperse in space via various biotic and abiotic vectors [15]. Characteristics of dormancy and dispersal, partly controlled by the hydrological regime, size, and spatial pattern of waterbodies, which, in term, are affected by human disturbance and climate change, are crucial for the successful long-term persistence of metapopulations and metacommunities of inhabitants of temporary waters [64]. By contributing to the knowledge of the biodiversity and spatial distribution of ostracods in Botswana, we hope to help address some of the knowledge gaps impeding the conservation of temporary waters in this country.

Note

Just when we received reviews of our manuscript, one more paper was published with records of ostracods from Botswana, which we could not take into account despite its relevance. Franchi et al. [65] found fossil valves of seven taxa of the species rank (five left in open nomenclature) from Late-Pleistocene-Holocene sediments of the Makgadikgadi Basin (Kalahari): *Candonopsis* sp., *Ilyocypris* sp., *Limnocythere* spp., *Potamocypris* sp., *Sarscypridopsis*

ochracea (Sars, 1924), Sarscypridopsis glabrata (Sars, 1924), and Sclerocypris cf. bicornis (Müller, 1900). Sarscypridopsis ochracea and Sclerocypris cf. bicornis are new records for Botswana.

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mój wkład polegał na udziale w opracowaniu wspólnej koncepcji pracy, oznaczeniu całości materiału, udziale w analizach numerycznych, sporządzeniu na podstawie danych z literatury katalogu gatunków wraz z aktualizacją taksonomiczną, danymi środowiskowymi i dotyczącymi położenia geograficznego stanowisk oraz przygotowaniu manuskryptu do druku.

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Article Evidence for Conductivity- and Macroinvertebrate-Driven Segregation of Ostracod Assemblages in Endorheic Depression Wetlands in North West Province of South Africa

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Abstract: Our knowledge of the ecology of non-marine Ostracoda inhabiting endorheic wetlands (pans) of the semi-arid regions of South Africa is very scarce. The present study investigates the distribution of ostracod species in grass, open, and salt pans in the central part of the North West province and tests ostracod response to abiotic and biotic predictor variables operating at a local scale. Distance-based linear models revealed three variables (pan type, water electrical conductivity and abundance of macroinvertebrate predators, and collector-gatherers) that best explained variation in the ostracod dataset. Ostracod assemblages from the three studied pan types differed by the dominance structure rather than by the species composition. Salt pans with high conductivity and high ratio of predaceous macroinvertebrates were dominated by *Heterocypris giesbrechti*, with accessory presence of *Plesiocypridopsis newtoni*. In open pans with low conductivities and the lowest ratio of predators (but highest ratio of collector-gatherers) *Potamocypris mastigophora* was typically a dominant species, while in grass pans, all the three mentioned species had similar relative abundances. Although our findings lend provisional support to some models of ostracod assemblage diversity across different pan types, more studies replicating endorheic depression wetlands in other regions are required before generalizations can be made.

Keywords: temporary pans; ostracod-environment relationship; semi-arid climate; predaceous macroinvertebrates

1. Introduction

The term "wetlands" groups a wide range of variable habitats, ranging between (semi-)terrestrial and aquatic systems, and which are distributed worldwide. Various factors, such as their hydrological cycle (permanent or temporary), substrate type, salinity, vegetation, or location, allow to distinguish different types amongst them. Owing to the delicate balance between rainfall and evapotranspiration, these areas are highly vulnerable to climate change [1]. Temporary waters, which are the most common type of wetlands in arid regions, are defined by Williams [2] as bodies of fresh water that experience a recurrent dry phase of varying length, making them one of the most unpredictable and seemingly unsuitable habitats in the world. Despite the fact that such waterbodies, commonly known as "pans" in South Africa [3], represent a significant part of the global landscape and have huge importance for the preservation of biodiversity, they are under threat from global climate change and a wide range of human activities [4,5].

One type of temporary waters are endorheic pans, which are characterized by circular to oval, sometimes kidney-like shape and a flat basin floor and by the fact that they



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Copyright: © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). only have inflow and no outflow channels [6]. During the dry season, endorheic pans can be completely dry, even for several years, until rain fall in the drainage area fills up these depressions in the terrain. The existence of these ecosystems is therefore largely dependent on general climate and specific weather conditions. In such highly variable environments, external factors strongly affect the development of the inhabitants of such temporary wetlands [7]. Invertebrates living in temporary waters are mainly exposed to desiccation, variation in water chemistry, high and strongly fluctuating temperatures, low oxygen concentrations, high light and UV radiation intensities, variation in a range of other environmental factors and habitat isolation [8]. In order to survive, such organisms must adapt to unsuitable environmental conditions by the acquisition of different life history strategies, for example by undergoing diapause or producing resting and dormant stages [9,10]. By producing resting eggs, organisms can also disperse passively, which is an adaptation to rapid colonization of new waterbodies and also to survive periods of drought and to allow development after another flooding [11,12].

Nhiwatiwa and Dalu [13] suggested that physical and chemical properties of water in temporary ecosystems play a fundamental role in shaping their communities, much more so than in permanent systems. For example, rapid water loss by evaporation can cause high levels of salinity in many endorheic wetlands [14].

Various groups of invertebrates abound in temporary waters, and ostracod assemblages tend to be particularly common and species-rich [15,16]. This is especially so for species of the family Cyprididae Baird, 1845 [17] which are generally excellent swimmers and can also be relatively large (the South African temporary pool ostracod *Megalocypris princeps* Sars, 1898 can be up to 8 mm long). This "gigantism" is largely related to the absence of fish, which predate on ostracods, in such temporary and/or saline water bodies, although invertebrate predation can also be significant (see below). The Cyprididae are, with more than 40% of all known species, the largest family of living non-marine ostracods [18]. Most of these species are free-swimming, produce drought resistant stages, and can often reproduce parthenogenetically. These three biological traits make cypridid species excellent dispersers. Yet, only few species have a cosmopolitan distribution and most are restricted to one (sub-)continent [18]. Several geographical regions have high levels of local endemicity of ostracods, and southern Africa is a well-known example [19]. This suggests that local environmental conditions can have a strong impact on the composition of communities and assemblages [20,21].

Here, we aim to determine the taxonomic composition and dominance structure of ostracod assemblages and also to examine how abiotic and biotic environmental factors influence such assemblages in endorheic waters of the central part of the North West province in South Africa. The novelty of this study is to address the role of macroinvertebrates and their functional feeding groups on ostracod assemblage distribution patterns.

2. Materials and Methods

2.1. Study Area, Location, and Selection of the Sampling Sites

The study sites were situated in the highveld plain (elevation of ca. 1350 m) of the central part of the North West province of South Africa (Figure 1), between Delareyville and Deelpan, where numerous generally shallow and non-perennial wetlands of various sizes occur and function as important breeding and feeding habitats for waterfowl and for aquatic invertebrates [22,23], but also as watering holes for game and cattle. This mostly agricultural area of ca. 300 km² belongs to an austral summer rainfall region located in a hot semi-arid zone which experiences erratic rainfall ranging from 360 to 560 mm per year, with the highest peak in January. The annual average temperature is 19–23 °C; however, extremely high monthly and daily temperature variation is typical for the area, with a minimum of around 0 °C in July and a maximum of 30 °C in January [23–27]; Ramsar Convention's web site http://www.ramsar.org (accessed on 27 June 2022). As a result of high evaporation rates, reasonably high concentrations of salts occur in the soil, thus sediments of depressional wetlands tend to be alkaline [23,24].



Figure 1. Map of the study area showing locations of the 16 sampling sites in the central part of the North West province of South Africa.

The sampling sites were chosen to include a relatively wide range of environmental conditions (regarding pan type and size, water chemistry or land use and human impact in the immediate catchment) in a rather small spatial extent in order to minimize the influence of environmental or climatic processes, operating at a more regional scale, on aquatic invertebrate communities.

In total 20 sites were sampled during two surveys. During the first survey, on 8 September 2009, samples from four sites localized close to each other in the Godwit Bay of Barberspan (between $26^{\circ}35'39''$ S, $25^{\circ}33'33''$ E and $26^{\circ}36'08''$ S, $25^{\circ}34'07''$ E) were collected for a reconnaissance faunistic and taxonomic study.

The second survey (at the end of the rainy season between 31 March and 5 April 2011) on ostracod-environment associations investigated 16 sites (Table 1, Figure 1) of which 15 were endorheic depressional wetlands or pans of three types (classified according to [28]): grass (8 sites), open (3 sites), and salt pans (4 sites). The remaining site was an artificial trough holding water for sheep (indicated as "other" in multivariate analysis), and was included in the present study to test if its ostracod assemblages were similar to those found in the studied natural pans. Except large (surface of almost 20 km² depending on the season) and perennial Barberspan, the other studied pans were temporary and reasonably small ($<5 \text{ km}^2$), as is the case for the majority of the pans in the North West province. Barberspan was originally also non-perennial but in 1918 water from the nearby Harts river was artificially diverted into Barberspan, changing the system into a permanent waterbody ([26] and Ramsar Convention's web site http://www.ramsar.org (accessed on 27 June 2022)). The studied sites were located mostly in relatively unimpacted natural areas (12 sites) and represented principally small (9 sites of surface area <0.2 km²), temporary waterbodies devoid of fish (10 sites) with sandy or sandy-muddy substrate (12 sites) and rather overgrown with macrophytes (Table 1).

Site	Longitude E	Latitude S	Altitude (m A.S.L.)	Water Temperature (°C)	pН	Electrical Conductivity (µS/cm)	Pan Type	Land Use	Fish Presence (Yes/No)	Macrophyte Coverage (%)	Substrate Type	Surface Area (km²)
1	26°20'39.00''	$25^{\circ}35'42.00''$	1347	27.5	10.0	10,670	salt	urban/degraded	Ν	10	muddy	0.97
2	26°20′43.00″	25°36′49.00″	1354	26.7	8.5	2670	grass	urban/degraded	Ν	40	sandy-muddy	< 0.01
3	26°23′59.00″	25°36′46.00″	1353	22.4	9.5	16,760	salt	natural	Ν	0	muddy	0.73
4	26°24′26.47″	25°36′10.02″	1357	29.3	8.5	358	grass	natural	Ν	90	sandy	0.08
5	26°25′16.00″	25°31′32.00″	1371	25.1	10.0	1123	open	urban/degraded	Ν	25	muddy	0.26
6	$26^{\circ}26'42.00''$	25°32′20.00″	1372	25.8	7.0	36	open	urban/degraded	Ν	0	sandy-muddy	0.05
7	26°26′29.00″	25°37′13.00″	1345	21.4	8.5	2700	grass	natural	Ν	40	sandy	0.56
8	26°30'11.25"	25°36′34.61″	1354	32.7	8.5	3900	salt	natural	Y	70	sandy	0.11
9	26°30′59.10″	25°36′32.92″	1354	32.7	8.5	3900	salt	natural	Y	70	sandy	4.05
10	26°32′54.00″	25°35′51.00″	1344	22.8	8.0	1109	grass	natural	Y	95	sandy-muddy	0.04
11	26°33'08.00''	25°36′02.00″	1358	25.9	7.5	1112	grass	natural	Y	95	sandy-muddy	0.04
12	26°33'19.00''	25°36'32.00''	1357	27.7	8.5	3010	grass	natural	Ν	80	sandy	0.01
13	26°35′28.70″	25°36′11.08″	1347	22.3	8.5	1101	grass	natural	Y	45	sandy	17.3
14	26°36′38.00″	25°34′54.00″	1347	22.5	7.0	519	grass	natural	Y	90	sandy-muddy	1.47
15	26°34′23.00″	25°33′11.00″	1371	19.1	7.0	1165	open	natural	Ν	75	sandy	0.04
16	26°26'30.00''	25°37′44.00″	1360	25.4	9.5	512	other	natural	Ν	0	concrete/artificial	< 0.001

Table 1. Data on geographical location, water properties, and other environmental characteristics of the sites from where ostracods and other invertebrates were collected for the present study in the North West province of South Africa.

2.2. Sampling, Identification of Biota, and Environmental Characterization

Biotic (ostracods and accompanying invertebrates) semi-quantitative samples were collected using a hand-net (120 μ m mesh size) from the bottom surface of ca. 0.5 m² (wherever possible) at depths to max. 50 cm, and then rinsed and preserved in 96% ethanol. Ostracods were sorted, identified, and counted using a stereoscopic light-reflected microscope at up to 25× magnification and a light-transmitted microscope at up to 400× magnification. Empty valves were not included in later analyses. Soft parts of ostracods were studied after full dissection with needles in glycerine on glass slides and valves were stored dry in micropalaeontological slides. Carapaces and valves were also observed and illustrated using scanning electron microscopy (SEM). Ostracods were identified down to the species level using primarily the following taxonomic literature [19,29–33]. The names of the ostracod species, their authorities, and family assignments are allocated according to a recent checklist [18]. Other invertebrates were identified mostly to the family level using guides by [34–43]. To each macroinvertebrate taxon, a Functional Feeding Group (FFG) was assigned as described in [41,42,44–49] and/or in the general classification system for aquatic macroinvertebrate FFG by [50].

At each site, a set of environmental variables were measured or assessed, including water properties, sediment and vegetation characteristics, site geography, and urban proximity. Longitude, latitude, and altitude (m A.S.L.) were recorded using a MLR Electronique SP24XC GPS receiver. Electrical conductivity (μ S cm⁻¹) at 25 °C, pH, and surface water temperature (°C) were measured in situ using a hand-held multi-parameter probe WTW Multi 350i. Type of substrate was categorized visually according to the dominant grain size fraction into muddy, sandy-muddy, sandy, or artificial. We also noted the perceived influence of urban activities and differentiated between two main types of study sites: (1) urban or degraded sites when pans were situated adjacent to or surrounded by towns or villages, and (2) natural sites when pans were relatively unaffected by urban activities. Data on fish presence were taken from the literature [26] and/or validated by field observation and confirmed by local landowners and residents (fish can be assumed absent in isolated (not connected to rivers and streams), small, temporary pans). An assessment of the total macrophyte vegetation cover at the sampling site was made visually and scored using an arbitrary scale, where 0: 0–10% vegetation cover of the water surface, 1: 11–50% and 2: 51-100%. The size of the studied waterbodies was calculated using GPS data and scored using a scale from 1 to 3 (where 1: <0.2 km², 2: 0.3–1.0 km² and 3: 1.1–20 km²).

2.3. Linking Ostracod Assemblage Analysis to Environmental Variables

The ostracod data included relative abundances (percentages) of species found at 13 out of 16 sampling sites of the 2011 survey. All analyses were based on the Bray-Curtis sample similarity matrix and performed with PRIMER 7 software [51] with the PERMANOVA+ add-on package [52].

A shade plot was used to determine patterns of ostracod assemblage groupings and to illustrate the distribution of individual species across different pan types. In the shade plot, samples (constrained by the pan type) and species were independently placed in the nearest neighbor order using the "greedy traveling salesman algorithm" (for details see [51,53]), based for samples on Bray-Curtis similarities on percentages, whereas for species on the association index on species-standardized data. Differences in ostracod assemblage composition between the three pan types were tested by Analysis of Similarity (ANOSIM). The Similarity Percentage (SIMPER) procedure was performed to determine which ostracod species contributed the most to the similarity among sites within each pan type and to the dissimilarity between the three pan types. To test if similarities between ostracod assemblages at the study sites correlate with the geographical distance between the sites, the RELATE routine was used by calculating a rank correlation coefficient between all the sites of their respective (dis)similarity/distance matrices (an analogue technique to parametric Mantel test, see for details [51,53]).

To test the relationship between ostracod-derived response dataset and environmental predictor variables, the distance-based linear model (DistLM) was used with a modified Akaike Information Criterion corrected for small sample size (AICc) [52] as selection procedure and "Best" as selection criterion. Significance of individual environmental variables responsible for the ostracod multivariate data was checked by the marginal tests, while the best fitted DistLM model was visualized and interpreted through a two-dimensional ordination tri-plot using the distance-based redundancy analysis (dbRDA) with overlying vectors of both predicting environmental variables and response ostracod data.

We used in total 10 explanatory variables of various nature in attempting to explain the ostracod assemblage distribution patterns (four continuous variables, five semi-quantitative or ordinal variables and one categorical variable): 1. Water electrical conductivity (continuous variable transformed to reduce skewness); 2. Water pH (continuous variable); 3. Substrate type (ordinal variable with four categories: muddy, muddy-sandy, sandy and concrete); 4. Land use (or human impact) in the immediate catchment (ordinal variable with two categories: natural and urban sites); 5. Fish presence (ordinal variable with two categories: absence and presence); 6. Macrophyte vegetation coverage (ordinal variable with three categories: 0, 1 and 2, see above on the used arbitrary scale); 7. Waterbody surface area (ordinal variable with three categories: 1, 2, 3, see above); 8. Pan type (categorical variable with 4 categories: grass, open, salt and other for the non-pan site). Two other continuous variables were biotic and derived from the macroinvertebrate datasets. First, we conducted principal component analysis (PCA) separately on percentage abundances of macroinvertebrate taxa and on percentage abundances of the macroinvertebrate FFGs. The sample scores of the first principal components (PC1 macroinvertebrate taxa and PC1 macroinvertebrate FFGs) were then saved as new variables and used as two additional variables (9 and 10) in DistLM and dbRDA analyses.

3. Results

3.1. Environmental Characteristics of the Studied Sites

Water temperature of the sampled sites during the 2011 survey ranged between 19.1 and 32.7 °C (mean \pm standard deviation = 25.6 \pm 3.85 °C) and depended on the time of the day, pH varied between 7.0 and 10.0 (mean \pm standard deviation = 8.4 \pm 0.98), while conductivity between 36 and 16,760 μ S cm⁻¹ (mean \pm standard deviation = 3165 \pm 4436.9 μ S cm⁻¹), showing considerably higher values in the salt pans. Environmental characteristics of the studied sites with in situ measurements of their water properties are provided in Table 1.

3.2. Regional Ostracod Diversity

A total of 28,088 specimens of ostracods belonging to 16 species were collected during the two surveys from 20 sites, 11,416 specimens of six species from four sites in the 2009 survey, and 16,672 specimens of 13 species from 16 sites in the 2011 survey. Three grass pans (nos. 2, 10 and 11, see Table 1) sampled during the 2011 survey did not yield any ostracods. All identified species with their authorities and taxonomic assignments to families are listed in Table 2, while scanning electron micrographs (SEM) of the recorded species are shown in Figures 2–4. Representatives of the family Cyprididae (13 species, 81% of the total species richness) and its subfamily Cypridopsinae (8 species, 50%) were the most species-rich taxa. In both surveys, *Heterocypris giesbrechti* (Figure 2A–H), *Plesiocypridopsis newtoni* (Figure 2I–M), and *Potamocypris mastigophora* (Figure 3A–C), were the most common species, each found at all four sites in the 2009 survey and at 9 sites (the two former species) or 7 (the latter one) out of 16 sites in the 2011 survey. On the other hand, as many as seven species richness at the individual sampling sites ranged from 1 (one site) to 5 (five sites) with the mean \pm standard deviation = 3.6 ± 1.3 .

Table 2. Preliminary checklist of living non-marine Ostracoda reported from North West province of South Africa. Included are only those published records which were identified down to species level or left in open nomenclature but containing specific epithet within a given genus. The records are listed in a taxonomically based order and in presently accepted taxonomic conventions following [18], although names as originally published are also provided following the source reference abbreviations. Sources: (1) [54] (Eliazar pan 25 km W Potchefstroom), (2) [55] (Eliazar pan 25 km W Potchefstroom), (3) [56] (Barberspan, a total of 8 species recorded but 7 left in open nomenclature not included here), (4) [57] (dolomitic springs in C-E North West), (5) [58] (dolomitic springs in C-E North West), (6) [59] (Molopo Oog), (7) [60] (Molopo Oog), (8) [19] (9) [61] (10) [62] (Molopo Oog), (11) [63] (Molopo Oog), (12) [64] (Molopo Oog), (13) [65] (Molopo Oog), (14) [66] (Ganalaagte pan, site no. 6 in the present paper), (15) present paper 2009 survey, (16) present paper 2011 survey.

Species	References						
Family Cyprididae	Baird, 1845						
Subfmily Cypridina	e Baird, 1845						
Pseudocypris expansa Sars, 1924	1,3						
Pseudocypris sp.	16						
Subfmily Cypridopsinae Kaufmann, 1900							
Plesiocypridopsis inaequivalva (Klie, 1933)	1 and 2 (as <i>Cypridopsis inaequivalva</i> Klie)						
Plesiocypridopsis newtoni (Brady & Robertson, 1870)	15, 16						
Potamocypris cf. deflexa (Sars, 1924)	16						
<i>Potamocypris</i> cf. <i>gibbula</i> (Sars, 1924)	15						
Potamocypris mastigophora (Methuen, 1910)	15, 16						
Potamocypris meissneri Szwarc et al. 2021	14, 16						
Sarscypridopsis aculeata (Costa, 1847)	15						
Sarscypridopsis elizabethae (Sars, 1924)	15						
Sarscypridopsis cf. katesae (Hartmann, 1957)	16						
Subfamily Cyprinotinae	e Bronstein, 1947						
Hemicypris congenera (Vávra, 1897)	1 (as <i>Cyprinotus congener</i> Vávra, doubtful						
	identification, may refer to Heterocypris						
	congenera (Vávra, 1897)),						
<i>Hemicypris</i> sp.	14 (as Hemicypris cf. inversa (Daday, 1913)), 16						
Heterocypris giesbrechti (G.W. Müller, 1898)	15, 16						
Subfamily Herpetocypridir	nae Kaufmann, 1900						
Chrissia levetzovi Hartmann, 1957	9						
Humphcypris greenwoodi Martens, 1997	5, 8						
Subfamily Megalocypric	linae Rome, 1965						
Sclerocypris exserta Sars, 1924	16						
Sclerocypris methueni (Kempf, 2015)	16						
Sclerocypris tuberculata (Sars, 1924)	1 (as Megalocypris tuberculata Sars)						
Family Ilyocyprididae	Kaufmann, 1900						
Ilyocypris cf. gibba (Ramdohr, 1808)	16						
Family Limnocytheri	dae Sars, 1925						
<i>Limnocythere</i> sp. ex gr. <i>stationis</i>	14 (as Limnocythere cf. stationis Vávra, 1897),						
	16						
Limnocythere cf. inopinata (Baird, 1843)	16						
Gomphocythere capensis G.W. Müller, 1914	4, 5, 10						
Family Darwinulidae Brad	y & Robertson, 1885						
Alicenula inversa (Martens & Rossetti, 1997)	6 (as Darwinula inversa), 7, 8						
Darwinula stevensoni (Brady & Robertson, 1870)	8						
Vestalenula molopoensis (Martens & Rossetti, 1997)	6 (as Darwinula molopoensis), 7, 8, 11, 12, 13						



Figure 2. SEM iconography of ostracods found at the studied sites in North West province of South Africa: *Heterocypris giesbrechti* (**A**–**H**); *Plesiocypridopsis newtoni* (**I**–**M**); *Hemicypris* sp. (**N**,**O**); *Limnocythere* sp. ex gr. *stationis* (**P**,**Q**); *Limnocythere* cf. *inopinata* (**R**,**S**). *H. giesbrechti*: (**A**) male, RV, external view. (**B**) male, LV, external view. (**C**) male, Cp, dorsal view. (**D**) female, Cp, right lateral view. (**E**) female, LV, external view. (**F**) female, Cp, dorsal view. (**G**) female, LV, internal view. (**H**) female, RV, internal view. (**I**) female, Cp, dorsal view. (**J**) female, RV, external view. (**K**) female, LV, external view. (**I**) female, Cp, dorsal view. (**J**) female, RV, external view. (**K**) female, LV, external view. (**I**) female, Cp, dorsal view. (**J**) female, RV, external view. (**K**) female, Cp, left lateral view. (**O**) female, Cp, dorsal view. *Limnocythere* sp. ex gr. *stationis*: (**P**) female, RV, external view. (**Q**) female, Cp, dorsal view. *L internata*: (**R**) female, Cp, right lateral view. (**S**) female, Cp, left lateral view. Scale = 500 µm for (N,O); 300 µm for (P,Q); 200 µm for (A–M); 100 µm for (R,S).



Figure 3. SEM iconography of ostracods found at the studied sites in North West province of South Africa: *Potamocypris mastigophora* (A–C); *Potamocypris* cf. *deflexa* (D,E); *Potamocypris meissneri* (F); *Potamocypris* cf. *gibbula* (G,H); *Sarscypridopsis aculeata* (I–L); *Sarscypridopsis* cf. *katesae* (M–O); *Sarscypridopsis elizabethae* (P–R). *P. mastigophora*: (A) female, LV, internal view. (B) female, RV, internal view. (C) female RV, external view. *P. cf. deflexa*: (D) female, Cp, left lateral view. (E) female, Cp, dorsal view. *P. meissneri*: (F) female, Cp, right lateral view. *P. cf. gibbula*: (G) female, Cp, left lateral view. (H) female, Cp, dorsal view. *S. aculeata*: (I) female, Cp, dorsal view. (J) female, Cp, right lateral view. (K) female, Cp, left lateral view. (L) female, Cp, ventral view. *S. cf. katesae*: (M) female, Cp, left lateral view. (Q) female, Cp, dorsal view. (R) female, detail of surface. *S. elizabethae*: (P) female, Cp, left lateral view. (Q) female, Cp, dorsal view. (R) female, detail of surface. Scale = 500 µm for (I–N,P,Q); 300 µm for (D,E,G,H); 100 µm for (A,B,F); 50 µm for (R); 20 µm for (C,O).



Figure 4. SEM iconography of ostracods found at the studied sites in North West province of South Africa: *Pseudocypris* sp. (**A**); *Sclerocypris methueni* (**B**–**D**); *Sclerocypris exserta* (**E**,**F**). *Pseudocypris* sp.: (**A**) female RV, external view. *S. methueni*: (**B**) female, Cp, dorsal view. (**C**) female, LV, external view. (**D**) female, RV, external view. *S. exserta*: (**E**) female, LV, external view. (**F**) female, RV, external view. Scale = 2000 µm for (**B**–**D**); 1000 µm for (**E**,**F**); 200 µm for (**A**).

3.3. Ostracod Species and Assemblage Distribution across Pan Types

The distribution and groupings of ostracods across different pan types sampled during the 2011 survey are illustrated in Figure 5. Statistically significant differences were found in the ostracod assemblage structure and composition between the pan types (ANOSIM Global test R = 0.459, p = 0.006) with significant distinction of the open pan ostracod assemblages which differed from those of salt pans (ANOSIM pairwise test R = 0.667, p = 0.029) and from grass pans (ANOSIM pairwise test R = 0.467, p = 0.036), whereas assemblages of salt and grass pans did not differ significantly (ANOSIM pairwise test R = 0.200, p = 0.087). The ostracod assemblages of the three studied pan types differed by the dominance structure rather than by the species composition, i.e., the average abundance of the three key species (the most common and abundant in the studied area H. giesbrechti, P. newtoni and P. mastigophora) were different in the salt, grass, and open pans (Table 3, see also Figure 5). Based on the SIMPER analysis, the average assemblage similarity within the salt pans was 66% and was made up mainly of contribution (almost 90%) from clearly dominating H. giesbrechti (with high average percentage abundance of 74%). Assemblages of grass pans were most similar to those of salt pans but showed low average similarity (23%) and all three species *H. giesbrechti* (average contribution of 57%), *P. newtoni* (27%), and *P. mastigophora* (14%) had similar average percentage abundances (21–24%). At the

open pan sites with average assemblage similarity of 30.5%, the total contribution was that of *P. mastigophora* (with high average abundance of 64%) (Table 3). However, one of the open pans (no. 6) was clearly distinct (Figure 5), hosting a very rare species, known so far only from this site, i.e., *Potamocypris meissneri* (Figure 3F) and a putative new species of the genus *Limnocythere* sp. (Figure 2P,Q). The ostracod assemblage found at the non-pan site (no. 16, a trough), with high abundance of *P. mastigophora*, was most similar (Bray-Curtis similarity of ca. 56%) to the assemblages from the open pans nos. 5 and 15 as well as from the grass pan no. 4 (Figure 5).



Figure 5. Shade plot illustrating the distribution and groupings of ostracods across different pan types in the North West province based on species percentage abundance shown in proportional shading intensity scale. Samples and species are independently placed in the nearest neighbor order, based for samples on Bray-Curtis similarities on percentages, and for species on the association index on species-standardized data. The sample axis is constrained by the pan type.

Table 3. Results of the SIMPER analysis listing ostracod species that contributed most to the average Bray-Curtis similarity (AvSim) among sites within each pan type and to the average dissimilarity (AvDiss) between all pair of sites among three pan types. For each species, the average percentage abundance (AvA) and individual percentage contribution (CSim/CDiss) to the average similarity/dissimilarity within/between the pan types are given.

	Salt Pans		Grass Pans		Open	Pans	Salt vs. Grass Pans	Salt vs. Open Pans	Grass vs. Open Pans
	AvSim :	= 66.36	AvSim = 23.34		AvSim = 30.54		AvDiss = 66.16	AvDiss = 99.66	AvDiss = 85.43
Species	AvA	CSim	AvA	CSim	AvA	CSim	CDiss	CDiss	CDiss
Heterocypris giesbrechti	74.09	89.65	24.04	56.77	0.00	0.00	37.99	37.17	14.07
Plesiocypridopsis newtoni	21.73	10.33	22.40	27.31	0.24	0.00	18.85	10.78	13.03
Potamocypris mastigophora	0.04	0.00	21.42	13.83	63.88	100.00	16.18	32.04	33.20

Finally, there was no significant correlation between site geographic distance and site assemblage similarity (RELATE statistics Rho = -0.078, p = 0.713).

3.4. Macroinvertebrate Communities and Functional Feeding Groups

The macroinvertebrate samples contained in total 1987 individuals belonging to 24 taxa, of which the majority (95%) belonged to six orders of insects (Coleoptera, Diptera, Ephemeroptera, Heteroptera, Lepidoptera, Odonata) (Table S1). True bugs of the suborder Heteroptera were the most abundant (34% of the total number of collected individuals), followed by Diptera (31%) with the most abundant and commonly occurring family Chironomidae (27%), and by Coleoptera (22%) with the second most abundant family Dytiscidae (19%) (Table S1). The first PCA axis (Table S3) of the macroinvertebrate taxa abundances explained 34.8% of the total variance, and was associated positively mainly with water boatmen hemipteran family Corixidae (0.877), and negatively with dipteran family Chironomidae (–0.424).

As regards functional feeding groups (FFGs), predators represented 63% of the macroinvertebrate taxa. This FFG included two families of Odonata and Coleoptera, three families of Diptera, and eight families of Heteroptera (Table S1). Oligochaeta, Ephemeroptera, and four families of Diptera identified as collectors were the second dominant FFG (25%) observed at the studied sites. The percentage abundance of each of the remaining three FFGs (filterers, shredders, and scrapers) was 4% (Table S1). PCA analysis using percentage abundances of FFGs as variables showed the highest positive correlation of collectors-gatherers (0.690) and negative correlation of predators (–0.719) with the first component PC1, which explained 85.3% of the total variance (Table S4).

Additionally, in the studied pans we found microcrustacean copepods of the families Cyclopidae and Diaptomidae as well as cladocerans of the families Macrothricidae and Moinidae (Table S1).

3.5. Effect of Environmental Variables on Ostracod Diversity and Assemblage Composition

Pan type, water conductivity, and FFGs PC1 were selected as significant environmental correlates of ostracod assemblage structure and composition (DistLM Marginal tests, p < 0.05, Table 4). These three variables each alone explained >20% of variation in the ostracod response dataset. The best two models based on the AICc criterion (with the lowest AICc value of 106.1–106.5 and correlation of 0.217–0.242) had one variable each: Conductivity or FFGs PC1. However, two-variable model (with conductivity and FFGs PC1), achieved nearly the same AICc value (106.9) as the two best models but had clearly higher correlation (0.382) (Table 4: Best models).

The best parsimonious two-variable DistLM model as a dbRDA ordination plot where the first axis accounted for 82.7% of the fitted model variation and 31.6% of the total variation is visualized in Figure 6. The first dbRDA axis is positively correlated with water electrical conductivity (0.656) and negatively correlated with FFGs PC1 (-0.755). It clearly separates the salt pans (located on the right side of the plot) with high water conductivity (mean \pm standard deviation = 8807 \pm 6118.1 µS cm⁻¹) and the highest ratio of predaceous (mean \pm standard deviation = 87.3 \pm 15.52%) but the lowest ratio of collector-gatherer (5.4 \pm 6.05%) macroinvertebrates from the remaining sites. This axis provides also some separation between the grass pans (mean electrical conductivity = 1538 \pm 1238.8 µS cm⁻¹ and mean percentage of predators = 67.5 \pm 23.41%) and the open pans (mean electrical conductivity = 775 \pm 640.0 µS cm⁻¹ and mean percentage of predators = 53.0 \pm 13.04%) as well as from the non-pan site. The second axis does not provide any division of the three types of pans. Although the first two dimensions captured 100% of the fitted model variation, there is still residual variation in the original data matrix since the two first axes together explain 38.2% of the total variation (Figure 6).

Table 4. Results of the distance-based linear model DistLM (marginal tests and five best parsimonious models) for the ostracod data from the North West endorheic wetlands using the best selection procedure and the modified Akaike Information Criterion (AICc) as selection criterion of environmental variables. R^2 = proportion of the variation in the ostracod dataset explained by environmental data, RSS residual sum of squares, FFGs PC1 = loadings of the first component of PCA on macroinvertebrate functional feeding groups.

Marginal Tests Variable	Sum of Squares (Trace)	Pseudo-F	Р	R ²
Pan type	19,113	2.703	0.013	0.4740
Conductivity	9762	3.514	0.014	0.2421
FFGs PC1	8747	3.047	0.030	0.2169
Macroinvertebrate PC1	6716	2.198	0.051	0.1666
Surface area	8696	1.375	0.209	0.2157
Substrate	11,679	1.223	0.257	0.2896
Fish presence	7312	1.108	0.345	0.1813
pH	3195	0.946	0.407	0.0792
Human impact	2266	0.655	0.622	0.0562
Vegetation cover	3694	0.504	0.881	0.0916
Best Models Variable Selection	No variables	AICc	RSS	R ²
Conductivity	1	106.11	30,562	0.2421
FFGs PC1	1	106.54	31,578	0.2169
Conductivity and FFGs PC1	2	106.93	24,926	0.3819
Macroinvertebrate PC1	1	107.35	33,608	0.1666
pH and FFGs PC1	2	108.21	27,502	0.3180



Figure 6. Ordination plot of the two first axes of distance-based redundancy analysis (dbRDA) depicting the best two-variable DistLM fitted model of significant relationships between ostracod dataset and environmental variables.

Of the three species selected by the SIMPER analysis as responsible for the pan type segregation, *Heterocypris giesbrechti* and *Plesiocypridopsis newtoni* are located on the right side of the plot (Figure 6) and are positively correlated with the dbRDA1 (correlation

coefficients 0.767 and 0.160, respectively). These species with high relative abundances are most characteristic for the salt (especially *H. giesbrechti*) and grass pans. On the other hand, *Potamocypris mastigophora*, which negatively correlated with the dbRDA1 (–0.256), is situated on the left side of the plot and occurred typically in high percentage abundances in the open pans.

4. Discussion

4.1. Ostracods of the North West Province

Whereas the non-marine ostracod fauna of South Africa (with the total reported species richness of ca. 120–125) has been relatively well-explored in the Western (ca. 60 species) and Eastern (43 species) Cape provinces as well as from KwaZulu Natal (ca. 50 species) [19,21,67], little appears to be known about the distribution of species and the structure of ostracod assemblages of the North West province (Table 2).

In the current study, 16 ostracod species were found, of which 13 have not been previously recorded from the North West (Table 2). One species has meanwhile been described as new from these collections, i.e., *Potamocypris meissneri* (in [66]) and three further species new to science (*Pseudocypris* sp., *Hemicypris* sp. and *Limnocythere* sp. ex gr. *stationis*) are currently being described. The present collections bring the total number of extant non-marine ostracod species recorded in the North West to 26 (Table 2) and show that further investigations may yield an even greater taxonomic diversity of ostracods in this region.

Species composition of the ostracod inventory of the North West known so far shows, as expected, that the general pattern of suprageneric taxonomic diversity with the most species-rich family Cyprididae (19 species, 73% of the total species richness of 26 species) and subfamily Cypridopsinae (9 species, 35%) is similar to that of the non-marine surface ostracod species inventories of the KwaZulu Natal (75% of species in Cyprididae and 21% in Cypridopsinae: [67]), of the Eastern Cape (77% species in Cyprididae and 26% in Cypridopsinae: [21]), as well as of Botswana (76% species in Cyprididae and 24% in Cypridopsinae: [68]). The most common and abundant ostracod species collected during the present study are widespread taxa which are not rare or threatened. *Heterocypris giesbrechti* has been found previously in North, West, East, and Central Africa [69–71] and occurs also in South Africa: in the KwaZulu Natal [67] and Eastern Cape [21]. *Potamocypris mastigophora* is also widely distributed across Sub-Saharan Africa (including South Africa) [19,69] with some records in southern Palaearctic [18,72], while *Plesiocypridopsis newtoni* occurs throughout Africa and beyond [18,73], and could constitute a Palaearctic introduction in South Africa. Other species were more limited in their distribution patterns in the studied area.

Nevertheless, the absence in our collection of a number of genera often recorded from various temporary wetlands in southern Africa, e.g., *Cypretta* Vávra, 1895; *Cypricercus* Sars, 1895; *Eundacypris* Martens, 1986; *Gomphocythere* Sars, 1924; *Globocypris* Klie, 1939; *Megalocypris* Sars, 1898; *Ovambocythere* Martens, 1989; *Physocypria* Vávra, 1897; *Ramotha* Martens, 1992 or *Zonocypris* Müller, 1898 (see [19,74]) shows that the fauna of temporary waters of this area can be regionally diversified, reflecting environmental specificity [75]. Significant differences in ostracod generic diversity (*Limnocythere* as one genus in common) between our study (9 genera in total) and that by [76] in pans of the Mpumalanga province of South Africa (6 genera) seem to support this hypothesis. The same appears true when comparing our results with those reported by [54] from three seasonal open mud pans and two grass pans in the Gauteng province and one open mud pan in the North West (the Eliazar pan already mentioned above). Of the total of 8 species found by these authors [54], only one (*Sarscypridopsis aculeata*) was also recorded in our study. Finally, of the six species reported from three temporary pans in central and northern Botswana [77], only one (*Potamocypris mastigophora*) was recorded in our study.

4.2. Ostracods-Environment Relationships

The results obtained in our study of the endorheic wetlands in the small area of the North West indicated that the three pan types supported diverse ostracod assemblages, differing mostly in the dominance structure and less in the species composition.

Considering that the three most common and abundant species recorded in the studied sites have relatively wide ecological tolerances [21,33,67,71,73,78–83], including conductivity, clearly other important, and possibly mutually correlated, drivers of non-random ostracod distribution and domination appeared to play a role. Indeed, even at the small local and habitat scale, different ostracod species may dominate in different environments, according not only to their tolerances to hydrochemistry (conductivity, pH, ionic ratios) or oxygen requirements, but also to their size, mode of life or vulnerability to predators, among others [84].

The relative abundance of predaceous and collector-gatherer macroinvertebrate functional feeding groups (expressed as the scores of PC1) was also selected as a significant environmental correlate of the ostracod assemblage structure and composition. Ostracod assemblages from the salt pans co-occurred with the highest ratio of predators. The grass pan assemblages appeared together with less abundant predators accompanied by collectors, whereas ostracods from the open pans were typically found with the lowest predator ratio, but the highest ratio of gathering collectors. In waterbodies without fish, several aquatic invertebrate taxa could prey on ostracods, including hemipterans or larval dytiscid beetles [84-86]. The ostracod assemblage structure may be impacted directly depending on predators specific selectivity and vulnerability of the prey. Several macroinvertebrate predators are size selective having clear preferences for smaller or larger ostracods [85,87]. Size-selective predation, which can be related to the predator specific trade-off between ease of detection and ease of capture of the prey, could not only affect the size distribution of specimens of individual species, but may also affect the dominance structure of an ostracod assemblage consisting of species differing in size. In the salt pans with the highest ratio of predators, consisting mostly of corixid and notonectid hemipterans, the dominant ostracod species was *H. giesbrechti*, which is the largest (ca. 0.79–0.90 mm in length, see [88]) of the three key species responsible of the ostracod assemblage separation among the studied pans (*P. newtoni* ca. 0.67–0.75 mm [73], while *P. mastigophora* ca. 0.52 [89]). On the other hand, in the open pans with the lowest ratio of predators, small *P. mastigophora* typically dominated. Predation pressure and size selectivity may certainly be reduced or modified by ostracod antipredator adaptations [85,90,91] as well as potential shifts in some life-history traits (as in cladocerans, see e.g., [92]). Our results did not allow to distinguish between such potential effects, but if (apart for size), several biological traits of the three most common species in the studied pans remain very similar (e.g., similar nektobenthic mode of life or no clear cryptic coloration), predator size-selectivity needs to be considered as a potential driver, structuring ostracod assemblages in pans of the study area, especially on relatively small spatial scales with low water depth and absence of vegetation where potential for vertical migration and hiding in plants is very limited.

In our study percentage abundance of predators was negatively correlated with that of gathering collectors, so that in the open pans both groups were co-dominant. By using the same resources and/or disturbing the habitat, making resources more difficult to access, some gathering collectors may potentially be competitive with ostracods. Modig et al. [93] showed experimentally that by the mechanical disturbance of sediment which may have caused a burial of phytodetritus, amphipods had negative effects on the feeding rate by ostracods on diatoms.

On the other hand, if the taxon richness and proportion of predatory macroinvertebrates in temporary habitats are positively correlated with the hydroperiod length (e.g., [11,94,95]), the relationship between the ratio of predators/collector-gatherers and ostracod assemblage structure revealed in our study may be also indirectly related to the hydroperiod. The significance of habitat permanence in structuring freshwater invertebrate communities has long been known [11], and has also been shown to be one of the important variables (based on invertebrate communities) in the segregation of reproductive modes (geographical parthenogenesis) in the Holarctic ostracod species *Eucypris virens* [96]. Whether the studied salt pans in the North West, harboring the highest proportion of predators and co-occurring ostracod assemblages dominated by *Heterocypris giesbrechti* are more stable, longer-standing waterbodies than those of the grass or open pans with other ostracod assemblages remains to be further studied. Our results suggest, however, that the biotic factors (principal components summarizing invertebrate functional feeding groups) may be more important in shaping ostracod assemblage structures than previously thought.

5. Conclusions

The current study extends the knowledge of the distribution of ostracods in South Africa, and describes for the first time the diversity in the structure of ostracod assemblages in different pan type habitats of the North West province, where biodiversity estimations have rarely included ostracods, skewing biodiversity assessments in favor of macroinvertebrates. By revealing that the pan type, water electrical conductivity, and abundance of macroinvertebrate predators and collector-gatherers best explained the variation in the ostracod dataset from the studied area, the present study provides a basis for continuing research on how ostracod assemblages vary in relation to both biotic (accompanying macroinvertebrates) and abiotic (water chemistry) environmental variables in endorheic wetlands and other similar habitats of other under-studied semi-arid areas of southern Africa. Identifying and understanding the drivers of the compositional structure of ostracod assemblages of temporary pans provide a baseline for further studies on the assessment of the impact of both climate change and direct anthropogenic disturbances on these endangered unpredictable ecosystems and facilitates application of ostracods in paleoenvironmental reconstructions. As evidenced by our collection of undescribed species and new records for the province, several species remain to be sampled (including new to science) from this part of southern Africa. Our scanning electron microscopy atlas may provide some help in identifying ostracods for further studies.

Supplementary Materials: The following supporting information can be downloaded at: https: //www.mdpi.com/article/10.3390/d15050614/s1. Table S1: Overview of ostracod species and other invertebrate taxa collected in 2011 from 16 study sites in North West province of South Africa. Abundances of individual taxa at the sites are presented as percentages. Names of macroinvertebrate taxa are accompanied by abbreviations of the functional feeding groups (FFGs) they represent: Cg—collector-gatherers, Ft—filterers, Pr—predators, Sc—scrapers/grazers, Sh—shredders. Table S2: Abundances of ostracod taxa collected in 2009 from 4 study sites in North West province of South Africa. Table S3: Results of the principal component analysis on relative abundances of the macroinvertebrate taxa accompanying ostracods collected in 2011 from 16 study sites in North West province of South Africa. Data on eigenvalues, explained portion of the total variation, and on coefficients of variables making up the first three PC's are shown. Macroinvertebrate taxa which correlated the most with the PC1 are highlighted in bold. Table S4: Results of principal component analysis on relative abundances of the macroinvertebrate functional feeding groups (FFGs) accompanying ostracods collected in 2011 from 16 study sites in North West province of South Africa. Data on eigenvalues, explained portion of the total variation and on coefficients of variables making up the first three PC's are shown. Macroinvertebrate FFGs which correlated the most with the PC1 are highlighted in bold.

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Table S1. Overview of ostracod species and other invertebrate taxa collected in 2011 from 16 study sites in North West province of South Africa. Abundances of individual taxa at the sites are presented as percentages. Names of macroinvertebrate taxa are accompanied by abbreviations of the Functional Feeding Groups (FFGs) they represent: Cg – collector-gatherers, Ft – filterers, Pr – predators, Sc – scrapers/grazers, Sh – shredders.

Site	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	Number of
Field code	SA06	SA07	SA11	SA03	SA08	SA09	SA10	SA02	SA01	SA12	SA14	SA16	SA04	SA05	SA18	SA17	individuals
Ostracoda																	
Pseudocypris sp.				1.0	0.2		0.6					69.0					289
Plesiocypridopsis newtoni	28.2		5.0		0.7		60.3	1.0	53.0			2.0	41.0	8.6			4461
Sarscypridopsis cf. katesae																44.0	726
Potamocypris cf. deflexa				0.2	5.8												75
Potamocypris mastigophora				83.8	91.6			0.2				14.0	9.0		100	56.0	3555
Potamocypris meissneri						65.8											217
Hemicypris sp.						0.6		5.7						80.0			136
Heterocypris giesbrechti	61.0		95.0	11.0			36.5	93.0	47.0			15.0	50.0	8.6			6750
Sclerocypris exserta	0.1				1.7			0.1									24
Sclerocypris methueni				4.0			2.6										179
Ilyocypris cf. gibba														2.8			1
Limnocythere cf. inopinata	10.7																148
Limnocythere sp.						33.6											111
Species richness	4	0	2	5	5	3	4	5	2	0	0	4	3	4	1		
Other microcrustaceans																	
Cyclopidae		100		100			100	78.0		100	100	100	96.6	36.5	33.3	100	368
Diaptomidae			100					22.0							66.7		17
Macrothricidae (<i>Macrothrix</i> sp.)						1.3								63.5			67
Moinidae (<i>Moina</i> sp.)						98.7							3.4				79
Macroinvertebrates																	
Oligochaeta (Cg)		1.8														40.7	64
Mollusca - Planorbidae (Sc)				30.8		3.4		0.4									41
Odonata - Coenagrionidae (Pr)		6.7								0.7		1.9					13
Odonata - Libellulidae (Pr)		7.9			3.7		0.3			3.6		3.8		1.6			31
Ephemeroptera (Cg)	3.1	9.1			5.6	6.7	3.0	12.4		9.3		30.2		2.0		0.7	108
Coleoptera - Dytiscidae (Pr)	20.3	0.6		51.7	14.1	54.0	18.8	6.6	4.0	4.3			7.3	53.3		1.3	371
Coleoptera - Hydrophilidae (Pr)	3.1	1.8			8.0	2.2	13.2					3.8	2.4	0.8	7.4		67
Hemiptera - Belostomatidae (Pr)	2.1	0.6			0.0		10.2			1.4		2.0		0.4			4
Hemiptera - Corixidae (Pr)	34.4	1.2	100	3.3	3.7	4.5	6.6	7.4	26.0	3.6	8.8	7.5	24.4	36.3		19.3	279
Hemiptera - Gerromorpha (Pr)	21		100	2.0	2.1		0.0	0.4	1.0	2.0	5.3			1.2		17.0	8
Hemiptera - Naucoridae (Pr)													24.4	0.4			11

Hemiptera - Nephomorpha (Pr)										3.6			4.9				7
Hemiptera - Notonectidae (Pr)	4.7			7.5	6.1			55.2	65.0			16.9			11.1		241
Hemiptera - Pleidae (Pr)		0.6		4.2	0.6	1.1	10.9	4.7		22.1		1.9	9.8	0.4			64
Hemiptera - Veliidae (Pr)		0.6			1.8		3.6	8.2	3.0	0.7	1.7		7.3	0.8	40.8		57
Diptera - Brachycera (Pr)	3.1						0.3										3
Diptera - Ceratopogonidae (Pr)							0.3			0.7				0.4			3
Diptera - Chironomidae (Cg)	4.7	69.1			56.4	28.1	37.3		1.0	50.0	84.2	30.2	14.7	2.0	37.0	16.0	527
Diptera - Culicidae (Ft)	25.0			2.5			3.0	3.9				1.9				21.3	71
Diptyera - Psychodidae (Cg)												1.9				0.7	2
Diptera - Stratiomyidae (Cg)							1.0	0.4									4
Diptera - Syrphidae (Cg)													2.4				1
Diptera - Tabanidae (Pr)	1.6						1.7	0.4					2.4	0.4			9
Lepidoptera - Pyralidae (Sh)															3.7		1
Total number of individuals	1447	166	1114	1881	1394	498	4353	2122	2754	246	123	443	417	392	32	1808	19190
% Ostracoda	96.0	0.0	98.4	89.5	88.0	66.0	92.9	87.5	96.0	0.0	0.0	80.0	83.0	8.9	6.2	91.6	
% Other microcrustaceans	0.0	0.6	1.2	4.1	0.0	16.0	0.1	0.4	0.0	43.0	54.0	8.0	7.0	26.5	9.4	0.1	
% Macroinvertebrates	4.0	99.4	0.4	6.4	12.0	18.0	7.0	12.1	4.0	57.0	46.0	12.0	10.0	64.6	84.4	8.3	

Таха	WM-5	WM-7	WM-9	WM-11
Plesiocypridopsis newtoni (Brady & Robertson, 1870)	706	1149	39	1318
Potamocypris cf. gibbula (Sars, 1924)	0	0	72	0
Potamocypris mastigophora (Methuen, 1910)	3	18	554	36
Sarscypridopsis aculeata (Costa, 1847)	1	207	489	531
Sarscypridopsis elizabethae (Sars, 1924)	0	0	0	151
Heterocypris giesbrechti (G.W. Müller, 1898)	289	1998	2817	1030
Sclerocypris sp. juv.	0	0	8	0

Table S2. Overview of ostracod species collected in 2009 from 4 study sites in North West province of South Africa (juv – juvenile).

Table S3. Results of Principal Component Analysis on relative abundances of the macroinvertebrate taxa accompanying ostracods collected in 2011 from 16 study sites in North West province of South Africa. Data on eigenvalues, explained portion of the total variation and on coefficients of variables making up the first three PC's are shown. Macroinvertebrate taxa which correlated the most with the PC1 are highlighted in bold.

Eigenvalues

	cintalaco		
ΡČ	Eigenvalues	%Variation	Cum.%Variation
1	883	34.8	34.8
2	636	25.1	59.9
3	485	19.1	79.0

Eigenvectors

LIGCINCCIOIS				
(Coefficients in the lin	lear combi	nations of	variables	making up PC's)
		0 002	0 1 2 0	
Oligochaeta	0.016	-0.003	-0.139	
Planorbidae	-0.046	0.122	0.206	
Libellulidae	-0.015	0.001	-0.018	
Coenagrionidae	-0.003	-0.004	-0.004	
Ephemeroptera	-0.081	-0.089	-0.018	
Hydrophilidae	-0.073	0.015	-0.100	
Dytiscidae	-0.140	0.637	0.576	
Veliidae	-0.116	-0.136	-0.143	
Notonectidae	-0.018	-0.734	0.491	
Corixidae	0.877	0.096	-0.175	
Pleidae	-0.030	0.010	-0.004	
Naucoridae	0.015	0.011	-0.052	
Belostomatidae	0.001	0.002	0.001	
Nephomorpha	0.003	0.002	-0.011	
Gerromorpha	0.003	-0.003	0.011	
Chironomidae	-0.424	0.061	-0.546	
Tabanidae	0.001	0.005	-0.006	
Culicidae	0.039	0.015	-0.043	
Psvchodidae	-0.003	-0.004	-0.006	
Stratiomvidae	-0.002	-0.001	-0.001	
Svrnhidae	0 001	0 001	-0.005	
Ceratonogonidae	0.000	0 002	0 001	
Brachycona	0.000	0.002	0.001	
Bi activel a	0.005	0.004	0.000	
Pyrailidae	-0.010	-0.008	-0.015	

Table S4. Results of Principal Component Analysis on relative abundances of the macroinvertebrate functional feeding groups (FFGs) accompanying ostracods collected in 2011 from 16 study sites in North West province of South Africa. Data on eigenvalues, explained portion of the total variation and on coefficients of variables making up the first three PC's are shown. Macroinvertebrate FFGs which correlated the most with the PC1 are highlighted in bold.

Eig	envalues		
PČ	Eigenvalues	%Variation	Cum.%Variation
1	1.17E+03	85.3	85.3
2	129	9.4	94.7
3	71.8	5.2	99.9

Eigenvectors

(Coefficients in the linear combinations of variables making up PC's)

Variable	PC1	PC2	PC3
Collector-gatherers	0.690	-0.512	0.078
Filterers	0.066	0.464	-0.733
Predators	-0.719	-0.479	-0.031
Shredders	0.003	-0.014	0.011
Scrapers/grazers	-0.041	0.541	0.675





Gdańsk, 5 czerwca 2023 r.

Oświadczenie

Oświadczam, że w pracy:

Szwarc A., Martens K., Meissner W., Namiotko T. 2023. Evidence for conductivity- and macroinvertebrate-driven segregation of ostracod assemblages in endorheic depression wetlands in North West Province of South Africa. *Diversity* 15 (5): 614. https://doi.org/10.3390/d15050614

mój wkład polegał na udziale w opracowaniu wspólnej koncepcji pracy, przeglądzie i wyborze literatury, oznaczeniu materiału, udziale w analizach numerycznych i interpretacji wyników, oraz na przygotowaniu manuskryptu, konsultacjach ze współautorami i edycji ostatecznej wersji manuskryptu.

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Statement TO WHOM IT MAY CONCERN

Brussels, 05 06 2023

I hereby declare that my contribution to the paper:

Szwarc A., Martens K., Meissner W., Namiotko T. 2023. Evidence for conductivity- and macroinvertebrate-driven segregation of ostracod assemblages in endorheic depression wetlands in North West Province of South Africa. *Diversity* 15 (5): 614. https://doi.org/10.3390/d15050614

includes assistance with the taxonomic identification of selected specimens, providing access to the museum collection of the reference material and to the scanning electron microscope facility as well as pre-editing of the manuscript.

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Gdańsk, 05.06.2023

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Oświadczenie

Oświadczam, że w pracy:

Szwarc A., Martens K., Meissner W., Namiotko T. 2023. Evidence for conductivity- and macroinvertebrate-driven segregation of ostracod assemblages in endorheic depression wetlands in North West Province of South Africa. Diversity 15 (5): 614. https://doi.org/10.3390/d15050614

mój wkład polegał na zebraniu części materiału badawczego oraz udziale w edycji pierwszej wersji manuskryptu.

/prof. dr hab. Włodzimierz Meissner/







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Oświadczenie

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Szwarc A., Martens K., Meissner W., Namiotko T. 2023. Evidence for conductivity- and macroinvertebrate-driven segregation of ostracod assemblages in endorheic depression wetlands in North West Province of South Africa. *Diversity* 15 (5): 614. https://doi.org/10.3390/d15050614

mój wkład polegał na zebraniu materiału badawczego, współudziale w opracowaniu koncepcji pracy, weryfikacji oznaczeń materiału oraz udziale w analizach numerycznych i w edycji tekstu.

KIEROWNIK Katedry Genetyki Ewolucyjnej i Biosystematyk prof. dr hab Tadeusz Namiotko



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On a new species of *Pseudocypris* Daday, 1910 (Crustacea, Ostracoda) from South Africa

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Abstract

The genus *Pseudocypris* Daday, 1910 has an Afrotropical distribution with only one species occurring in other region. A new species, *Pseudocypris* **sp. nov.**, collected from four temporary water bodies in North West province is a fifth species recorded from South Africa. The prominent diagnostic characters of a new species distinguishing it from other members of the genus are the shape of the carapace covered with numerous spines as well as the shape of hemipenis and prehensile palps. This paper also provides a detailed description of the copulatory organs and all appendages of the female and male together with SEM pictures of valves. We concluded that the genus *Pseudocypris* needs extensive taxonomic revision as well as comprehensive ecological studies.

Keywords: Afrotropical, biodiversity, new species, microcrustaceans, morphology, temporary waters

Introduction

Ostracods are small crustaceans which form an important part of the zoobenthos in African freshwaters. Of the approximately 2,300 species described worldwide, about 120 occur in South Africa (Martens 2001; Meisch et al. 2019). However, this is only a part of the total taxonomic diversity that can be expected in this area.

The subfamily Cypridinae comprise eight genera, among which *Pseudocypris* Daday, 1910 can be distinguished by a combination of such major diagnostic characters as: a) both valves with submarginal anterior selvage, b) at least left valve anteriorly with a large inner list, c) often serrated valve margins, d) exterior, lateral ala of various widths on valves of most species, e) second thoracopod with penultimate segment fused, f) seta "d2" completely missing and g) hemipenis generally with 6–8 "8"-shaped loops of the inner spermiducts (Martens 1990; Martens 2007). The genus *Pseudocypris* is distributed mostly in the Afrotropical region and only one species belonging to this genus, *P. patialaensis* Battish, 1977 was found in the Oriental region in India (Battish 1977). Although the type species, *P. bouvieri* Daday, 1910 was

described from Lake Rukwa in Tanzania, half of all known species have so far been recorded from South Africa (Methuen 1910; Sars 1924a, 1924b).

Until now, a complete description of the females has been lacking. Authors focused mainly on the male copulatory organs and traits of the carapace. Our study provides a detailed morphological description with illustrations of appendages and valves of both sexes of a new species *Pseudocypris* **sp. nov.** collected from temporary waterbodies in the North West province of South Africa.

Materials and methods

Samples were taken from four localities in the North West province in South Africa in March and April 2011. Ostracods were collected using a hand-net (mesh size of 120 μ m) to sweep the bottom surface and among vegetation at the depth of < 50 cm. In the field, sediment samples were rinsed, placed in plastic jars and preserved in 96% ethanol, whereas in the laboratory these were again rewashed with tap water through a 120 μ m-mesh sieve and preserved in fresh 96% ethanol. Specimens were sorted, counted and dissected using a binocular and light transmission microscope according to Namiotko et al. (2011). Soft parts were mounted in glycerin on glass slides, while valves were stored dry in cavity micropalaeontological slides. Drawings of soft parts were made with the aid of a camera lucida on a transmission light microscope Nicon Eclipse 50i (Univ. Gdansk, Poland). Carapace and valves were gold-coated and observed under the scanning electron microscope, Fei Qanta 200 ESEM, at the Royal Belgian Institute of Natural Sciences, Brussels, Belgium.

A hand-held multi-parameter probe WTW Multi 350i were used to measure water electrical conductivity and pH of the sampled pans.

The type material is deposited in the Ostracod Collection of the Laboratory of Biosystematics and Ecology of Aquatic Invertebrates, Department of Evolutionary Genetics and Biosystematics, University of Gdansk, Poland (OC-UG) and in the Collection of the Royal Belgian Institute of Natural Sciences, Brussels, Belgium (RBINS).

The model of limb chaetotaxy proposed by Broodbakker & Danielopol (1982) is followed, together with the revised version for the second antenna proposed by Martens (1987) and Scharf et al. (2020). Names for the limbs were used according to Meisch (2000).

Abbreviations used in text and figures

Limbs:

a, a'	two setae on Pr of T1
A1	first antenna (antennule)
A2	second antenna
alfa (a)	special seta on the 1 st podomere of Md palp
b	seta on Pr of T1
beta (β)	special seta on the 2 nd podomere of Md palp
CR	caudal ramus
d	seta on Pr of T1
d1, d2, dp	setae on Pr of T2 and T3
dls	dorsal lateral shield of hemipenis

Ε	endopod
e	setae on EI of T2 and T3
EI-EIV	1 st to 4 th podomeres of E
f	setae on EII of T2 and T3
g	setae on EIII of T2 and T3
Ga	anterior claw of CR
Gp	posterior claw of CR
gamma (y)	special seta on 3 rd podomere of Md palp
GM (Gm)	major (minor) claw on EIV of A2
G1–3	anterior and internal claws (or setae) on EIII of $\mbox{A2}$
Нр	hemipenis
h1-3	setae (or claws) on EIV of T2 and T3
Md	mandibula
ms	medial shield of hemipenis
Mx1	maxillula
ns	natatory setae on A2
Pr	protopod
S1-2	plumed setae on 1 st podomere of Md palp
Sa	anterior seta of CR
Sp	posterior seta of CR
T1	first thoracopod (maxilliped)
T2	second thoracopod (walking leg)
T3 t	third thoracopod (cleaning leg)
t1-4	internal setae on EII of A2
vls	ventral lateral shield of hemipenis
Y	aesthetasc on EI of A2
y2, y3	aesthetascs on EII and EIII of A2, respectively
ya	aesthetasc on the terminal podomere of A1
z1-3	external setae (or claws) on EII of A2

Valves and carapace:

Ср	carapace
H	valve height
L	valve length
LV	left valve
RV	right valve

Taxonomy

Class Ostracoda Latreille, 1802 Subclass Podocopa Sars, 1866 Order Podocopida Sars, 1866 Suborder Cypridocopina Baird, 1845 Superfamily Cypridoidea Baird, 1845 Pseudocypris sp. nov.

Material examined. Type locality: South Africa, North-West Province, small temporary grass pan (SA-16) near the Barberspan lake (see Szwarc et al. 2023 Fig. 1, site no. 12); 26°33'19"S, 25°36'32"E, elevation 1357 m a.s.l.; 4 Apr. 2011; T. Namiotko leg.

Holotype: • 1 \bigcirc (adult); dissected female stored on a permanent microscopic slide and valves stored dry in a micropalaeontological slide *Allotype*: • \bigcirc (adult); same collection data as for holotype *Paratypes*: • 16 \bigcirc \bigcirc (adults), 226 juv.; same collection data as for holotype; preserved in 96% ethanol; repository: OC-UG and RBINS. Accompanying ostracod fauna: *Heterocypris giesbrechi* (G.W. Müller, 1898); *Plesiocypridopsis newtoni* (Brady & Robertson, 1870); *Potamocypris mastigophora* (Methuen, 1910).

Additional material. South Africa, North-West Province: • SA-3: $4 \ Q \ Q$, $4 \ S \ d$ and 9 juv.; small temporary grass pan near the Witpan; 26°24'26"S, 25°36'10"E, elevation 1357 m a.s.l.; 31 Mar. 2011; T. Namiotko leg.; preserved in 96% ethanol; repository: OC-UG; accompanying ostracod fauna: *Heterocypris giesbrechi* (G.W. Müller, 1898); *Sclerocypris methueni* (Kempf, 2015); *Potamocypris mastigophora* (Methuen, 1910); *Potamocypris* cf. *deflexa* (Sars, 1924); • SA-8: 1 empty carapace and 2 juv.; endorheic open Kopelapan near the village of Kopela; 26°25'16"S, 25°31'32"E, elevation 1371 m a.s.l.; 1 Apr. 2011; T. Namiotko leg.; preserved in 96% ethanol; repository: OC-UG; accompanying ostracod fauna: *Plesiocypridopsis newtoni* (Brady & Robertson, 1870); *Sclerocypris exserta* Sars, 1924; *Potamocypris mastigophora* (Methuen, 1910); *Potamocypris* cf. *deflexa* (Sars, 1924); • SA-10: 1 Q and 25 juv.; grass pan near Hattingh Farm; 26°26'29"S, 25°37'13"E, elevation 1345 m a.s.l.; 2 Apr. 2011; T. Namiotko leg.; preserved in 96% ethanol; repository: OC-UG; accompanying ostracod fauna: *Plesiocypridopsis newtoni* (Brady & Robertson, 1870); *Heterocypris giesbrechi* (G.W. Müller, 1898); *Sclerocypris methueni* (Kempf, 2015).

Diagnosis.

Carapace in lateral view with dorsal margin slightly arched with greatest height situated in front of mid-length and sloping towards the posterior edge. Lateral ala c. 1/3 of the width of one valve, not equally wide along the circumference of the carapace in dorsal view. External surface delicately reticulate with large spines, especially in postero-dorsal areas. LV slightly overlapping RV posteriorly. A1 7-segmented, Rome organ not seen. A2 with long swimming setae. Md-palp with two claws and four setae, α -seta smooth, β - and γ -seta hirsute. Terminal segment od Mx1-palp elongate with five setae. Respiratory plates of T1 with six hirsute rays. Prehensile palps 2-segmented, left palp with terminal segment hook-like, long and narrow, right one with terminal segment wider and shorter than left one. Penultimate segments of T2 and T3 undivided. CR stout, well developed. Hp with pointed ms, dls small subrectangular, vls subtriangular, wide and large, dorsal margin sunken, proximally arched and beak-like apically.

Description. Female.

Cp in dorsal view (Fig. 1A-B) almost round with lateral ala c. 1/3 of the width of one valve, not equally wide along the circumference of the carapace. Ala protrudes on about 60% of the central part of the valve. Numerous spines at the top and around the circumference of the carapace. Greatest width situated insignificantly behind mid-length. LV slightly overlapping RV posteriorly. Cp in lateral view (Fig. 1C-D) subrectangular with anterior margin much more rounded than posterior one. Dorsal margin slightly arched with greatest height situated in front of mid-length and sloping towards the posterior edge. Ventral margin with almost straight ala. External surface delicately reticulate with large spines in postero-dorsal and ventral areas (Fig. 1E-H). Anterior margin of RV serrated.

A1 (Fig. 1C) 7-segmented. First segment with two long ventral setae (one of them broken) and one medium subapical dorsal seta. Second segment wider than long, with one short anterodorsal seta. Rome organ not seen. Third segment bearing two setae, one short antero-ventral and one medium antero-dorsal, the former reaching tip of the next segment, the latter longer than the fifth segment. Fourth segment with two long antero-dorsal setae and subequal antero-ventral setae, one short reaching almost tip of the next segment and one medium slightly longer than the sixth segment. Fifth segment with four long apical setae, one antero-ventral seta shorter than the other three. Penultimate segment with four long setae. Terminal segment bearing three (two long, one short) apical setae and aesthetasc ya, the latter c. twice as long as short apical seta. All long setae are plumose.

A2 (Fig. 2D) with protopodite, exopodite and 3-segmented endopodite. Basal segment of protopodite with two medium ventro-apical setae. Second segment of protopodite with one long subapical seta, reaching beyond the tip of the next segment. Exopodite reduced to a small plate with three setae, one long reaching beyond first endopodal segment, and two short setae. First endopodal segment with short aesthetasc Y, divided in three parts with elongated distal sensorial part; one medium plumose ventro-apical seta, exceeding beyond tip of the next segment and with five long antero-dorsal natatory setae, reaching tips of terminal claws and one shorter (6^{th}) seta reaching beyond the half of the next segment. Second endopodal segment divided, with four medio-ventral setae (t1-4), three long slightly serrated and one short; two unequally medio-dorsal setae, one long and one medium length. Distally with three slender z-setae: two medium length (z1, z2) and one long z3 and three serrated unequally G-claws: G2 slightly shorter than G1 and G3. Penultimate segment also with very short y2 seta. Terminal endopodal segment subquadrate, with a long serrated claw GM, a shorter (~ 2/3 length of GM) claw Gm and an aesthetasc y3 fused with considerably longer accompanying setae; smooth g-seta slightly longer than claw Gm.

Md-palp (Fig. 2A) 4-segmented. First segment with one long smooth, subapical seta (S2); ventro-apically with three setae, one long slender, one long plumed seta (S1) and medium length α -seta. Second segment ventrally with four almost equal long setae and medium length hirsute β -seta; antero-dorsally with three long slender and smooth setae. Penultimate segment consisting of three groups of setae: antero-dorsally with five long, but unequal apical setae; medio-dorsally with three long smooth setae and a stout and hirsute γ -seta; antero-ventrally with one long and one medium setae. Terminal segment bearing two claws and four setae.

Md-coxa (Fig. 2B) typically elongated, distally with rows of teeth and small setae, and with one short serrated seta situated near the insertion place of the palp.

Mx1 (Fig. 3C) with a 2-segmented palp and three endites (incompletely illustrated). Basal segment of palp with a group of five unequal setae (two long, two medium and one short); terminal segment elongated, c. three times as long as its basal width, apically with five setae (slightly longer than second palp-segment).

T1 (Fig. 3E) protopodite with two unequal short a-setae and long b-and d-setae (b-seta broken), c-seta absent; distally with apical setae of unequal length (incompletely illustrated). Endopodite elongated, apically with three setae, one long (h2 broken) and two shorter (h1, h3). Respiratory plates with six hirsute rays of differing lengths.

T2 (Fig. 3D) a walking limb. Protopodite with seta d1 (broken), seta d2 absent. First endopodal segment with one short seta e. Penultimate segment fused with two setae, medially medium length f seta and apically short g seta, the former reaching middle of next segment, the latter reaching beyond distal end of fourth endopodal segment. Terminal segment with one smooth seta (h3) and two claws, one short hirsute (h1) and one long distally serrated (h2).

T3 (Fig. 3A) a cleaning limb. Protopodite with three long setae (d1, d2, dp). First endopodal segment with long subapical hirsute seta e, reaching almost tip of the next segment. Second and third endopodal segments fused, bearing short hirsute f-seta, reaching tip of the segment. Terminal segment, a pincer organ, with medium length seta (h3), a short curved seta (h2) and a very short seta (h1), length of seta h3 ~ 2/3 that of penultimate segment, seta h2 ~ 1/4 length of seta h3.

CR (Fig. 3B) stout. Anterior and posterior claws serrated, length of posterior one c. 1/2 of that of anterior claw. Posterior seta slightly longer than anterior one, reaching beyond tip of ramus.

Measurements (in μ m). Cp: L = 2291, W = 2197; LV: L = 2205, H = 1313; RV: L = 2218, H = 1348.

Male.

All limbs as in female, except for last two segments of A2, T1 and reproductive organs. Penultimate segment of A2 (Fig. 4A) with claw-like z1 and z2 setae, significantly reduced serrated claw G1 and with claw G3 reduced to thin setae; terminal segment with claw GM considerably reduced to thin setae (~ 1/3 the length of Gm) and large serrated claw Gm. Plumose ventro-apical seta on first endopodal segment of A2 much more longer than the female seta.T1-endopodite forming asymmetrical prehensile palps; left palp (Fig. 6B) with terminal segment hook-like, long and narrow. Right palp (Fig. 6C) with terminal segment wider and shorter than left one. Both prehensile palps with two unequal apical spines on subrectangular basal segment. Hemipenis (Fig. 6A) with ms pointed, dls small subrectangular, vls subtriangular, wide and large, dorsal margin sunken, proximally arched and beak-like apically.

Ecology.

Pseudocypris **sp. nov.** was found in three natural grass pans and one open pan in the North West province in South Africa. This species occurs in waterbodies of conductivity ranging 358-3010 μ S/cm and pH ranging from 8.5 to 10.0.
Discussion

Pseudocypris sp. nov. is the fifth species of this genus found in South Africa. Most of the species are poorly described and the morphology of the soft parts is partly known. The distinction between species is mainly based on the shape of the valves and male reproductive organs. The new species is the most similar to Pseudocypris spinosa Methuen, 1910 due to the presence of spines on the valves, which in the latter are much more prominent. Differences are also visible in the shape of male copulatory organs. P. spinosa has straight dorsal margin of vls and more rounded ms of hemipenis. The basal parts of both prehensile palps are larger in the newly described species and the left one is more slender, while the terminal part of the right one is much more curved. Additionally, P. spinosa has never been found outside the direct range of its type locality, Lake Chrissie area, which may suggest that it is an endemic species (Methuen 1910). Apart from the new species, the occurrence of serrated RV margin was recorded only for P. gibbera Sars, 1924, characterized by the absence of lateral ala. Pseudocypris sp. nov. has hemipenis with very pointed and boot-like ms as well as wide and large vls with dorsal margin sunken. The shape of this organ distinguishes this species from other congeners, especially from the P. circularis Sars, 1924, which has a vls more rounded. P. acuta G.W. Müller, 1914 has hamipenis with almost straight distal part of ms, and vls large and elongated unlike *P. bouvieri* whose hamipenis is smaller and has rounded heel. Whereas P. gibbera has more squarish ventral extremity of ms and shorter vls. The hemipenis shape of Pseudocypris sp. nov. is most similar to P. expansa and P. triquetra Sars, 1924. The former can be distinguished by straight distal margin of vls and broad but not pointed ms, while the latter has elongated ventral tip of vls and ms with rounded lobe (Sars 1924a, 1924b; Martens 1990). The combination of spines on the valves, serrated RV margin and shape of hemipenis clearly distinguishe a new species described here from other *Pseudocypris* species.

The occurrence of *Pseudocypris* **sp. nov.** in the North West province of South Africa confirms the southern African distribution pattern of species belonging to this genus. Of all eight known species from Afrotropical region, only *P. gibbera*, *P. circularis* and *P. bouvieri* were found outside the South Africa. The first two were listed in Botswana, Namibia and Zimbabwe (Sars 1924b, Barnard 1935, McKenzie 1971, Martens 1990, Szwarc et al. 2022), while the last one in Tanzania and Kenya (Daday 1910, Rumes 2016).

The genus *Pseudocypris*, widespread in southern Africa, requires extensive taxonomic revision, including a detailed description of the soft parts of both males and females, as well as comprehensive ecological studies that may explain the influence of environmental conditions on the occurrence of the representatives of this genus.

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Figure 1. Carapace and valves of *Pseudocypris* **sp**. **nov**. \bigcirc . **A.** Cp, dorsal view. **B.** Juvenile, Cp, dorsal view. **C.** RV, external view. **D.** LV, external view. **E.** RV, detail of poster-dorsal part of C showing the spines. **F.** LV, detail of poster-dorsal part of D showing the spines. **G.** Cp, dorsal view, detail of posterior part of A showing the spines. **H.** LV, detail of ventral part of D showing the spines. Scale = 2000 µm for A; 1000 µm for B-D; 500 µm for G; 400 µm for E-F, H. Arrow indicates anterior direction.



Figure 2. Limbs of *Pseudocypris* **sp**. **nov**. \bigcirc . **A.** Mandibular palp **B.** Mandibular coxa **C.** First antenna **D.** Second antenna. Scale bars: 100 μ m.



Figure 3. Limbs of *Pseudocypris* **sp. nov**. \bigcirc . **A.** Third thoracopod (cleaning leg) **B.** Caudal ramus **C.** Maxillula **D.** Second thoracopod (walking leg) **E.** First thoracopod (maxilliped). Scale bars: 100 µm.



Figure 4. Limbs of *Pseudocypris* **sp**. **nov**. \mathcal{J} . **A.** Second antenna **B.** First antenna **C.** Mandibular palp **D.** Mandibular coxa. Scale bars: 100 μ m.



Figure 5. Limbs of *Pseudocypris* **sp. nov**. \mathcal{J} . **A.** Caudal ramus **B.** Third thoracopod (cleaning leg) **C.** Second thoracopod (walking leg) **D.** Maxillula **E.** Maxillular respiratory plate **F.** Foodrake **G.** First thoracopod (maxilliped). Scale bars: 100 µm.



Figure 6. Limbs of *Pseudocypris* **sp**. **nov**. \mathcal{O} . **A.** Hemipenis **B.** Left prehensile palp **C.** Right prehensile palp. Scale bars: 100 μ m.





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Oświadczenie

Oświadczam, że w manuskrypcie:

Szwarc A., Namiotko T. On a new species of *Pseudocypris* Daday, 1910 (Crustacea, Ostracoda) from South Africa

mój wkład polegał na udziale w opracowaniu wspólnej koncepcji pracy, oznaczeniu materiału, wykonaniu trwałych preparatów osobników typowych, a także przygotowaniu treści manuskryptu, mapy terenu badawczego oraz rysunków części miękkich opisanego gatunku i tablic ze zdjęciami skorupek spod elektronowego mikroskopu skaningowego.

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mój wkład polegał na zebraniu materiału badawczego, udziale w wypracowaniu wspólnej koncepcji pracy, wstępnym oznaczeniu taksonomicznym materiału oraz naniesieniu poprawek w tekście.

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