# Double blow: Alien crayfish infected with invasive temnocephalan in South African waters

**AUTHORS:**
Louis du Preez[1]
Nico Smit[1]

**AFFILIATION:**
[1]Unit for Environmental Sciences and Management, Subject area Zoology, North-West University, Potchefstroom, South Africa

**CORRESPONDENCE TO:**
Louis du Preez

**EMAIL:**
Louis.duPreez@nwu.ac.za

**POSTAL ADDRESS:**
Unit for Environmental Sciences and Management, Subject area Zoology, North-West University, Private Bag X6001, Potchefstroom 2530, South Africa

**DATES:**
**Received:** 16 Apr. 2013
**Revised:** 29 May 2013
**Accepted:** 30 May 2013

**KEYWORDS:**
alien crayfish; aquatic health; South Africa; *Cherax quadricarinatus, Diceratocephala boshmai*

**HOW TO CITE:**
Du Preez L, Smit N. Double blow: Alien crayfish infected with invasive temnocephalan in South African waters. S Afr J Sci. 2013;109(9/10), Art. #2013-0109, 4 pages. http://dx.doi.org/10.1590/sajs.2013/20130109



Trade in live, freshwater crayfish for ornamental markets, as well as for aquaculture, has grown rapidly and has become the major pathway for the introduction of non-indigenous crayfish species to several countries worldwide. Here we report on the first record of the Australian 'redclaw' *Cherax quadracarinatus* in the natural waters of a game reserve in South Africa. To compound the situation, these redclaw crayfish were infected with a non-indigenous temnocephalan flatworm parasite. Both crayfish and temnocephalan were in full breeding condition, with young. Further spreading of this crayfish to the subtropical, water-rich, northern KwaZulu-Natal Province of South Africa and southern Mozambique is predicted. Not only might the crayfish compete with indigenous aquatic invertebrates but the non-host-specific temnocephalan might transfer to local decapods, such as freshwater crabs.

## Introduction

Freshwater crayfish imports for aquaculture, especially those from Australia, have established alien populations of these crustaceans in several countries.[1,2] At least four crayfish species have at some stage been imported, or been considered for importation, into South Africa.[3,4] These include the 'redclaw' (*Cherax quadricarinatus* van Martens), the 'yabby' (*Cherax destructor* Clark), the 'marron' (*Cherax tenuimanus* (Smith)) and the 'red swamp crayfish' (*Procambarus clarkii* (Girard)).

Based on the number of permit requests to import these species into South Africa, as well as the documented opposition to these applications, De Moor[3] compiled a review of the potential impact of these species on South African freshwater ecosystems. According to De Moor[3], of these potentially invasive species, naturalised populations exist for *P. clarki* at an impoundment near Dullstroom on the Crocodile River[4] and for *C. quadricarinatus* in Swaziland at the Sand River Dam, situated on a tributary of the Crocodile River. However, the Sand River Dam in Swaziland is sited on a tributary of the Komati River and not the Crocodile River as indicated by De Moor[3] and later by Picker and Griffiths[5] (Figure 1).

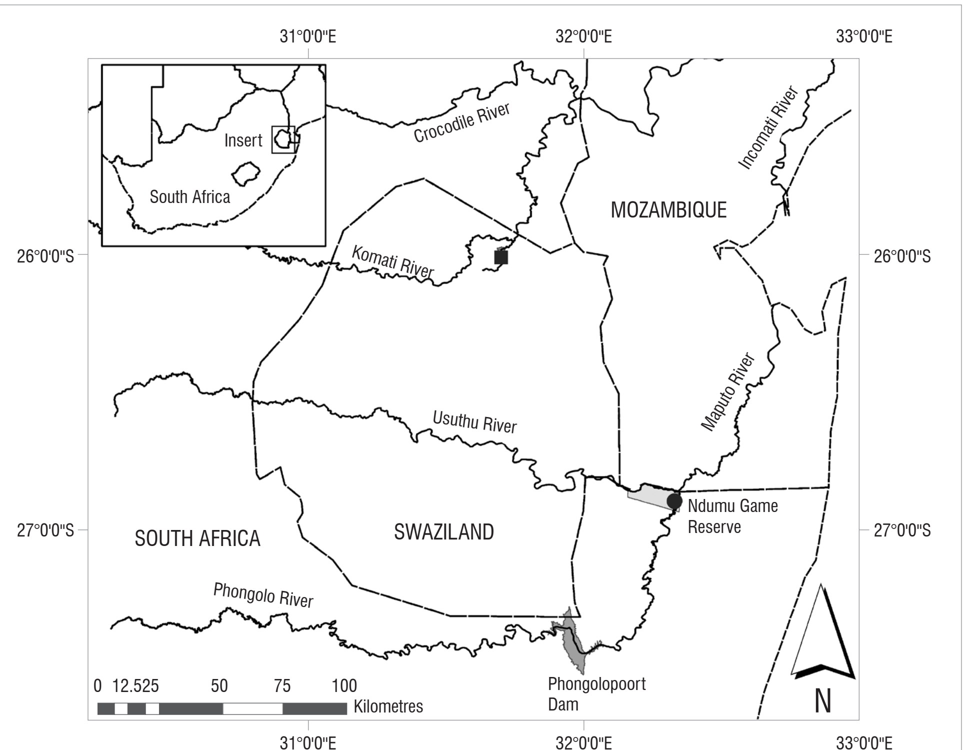



**Figure 1:** Map showing the location of a previously reported alien population of *Cherax quadricarinatus* (square) in the Sand River Dam in Swaziland[11] and the current record (circle) of the same species at the outlet of Lake Nyamiti (26.882775 S, 32.311393 E) in the Ndumu Game Reserve, KwaZulu-Natal.

In the case of the Swaziland population of *C. quadricarinatus*, it has been reported that a breeding colony has been established and that the crayfish are spreading into irrigation canals.[3] Establishing alien populations in new localities seems to be the norm for introduced *C. quadricarinatus* as wild populations have also recently been reported from

Puerto Rico[6], Mexico[1], Singapore[2,7], Zambia[8] and Israel[9]. Interestingly, although both Ahyong and Yeo[2] and Belle and Yeo[7] mentioned the alien populations of *C. quadricarinatus* in South Africa, the references these authors quoted – Zimmerman[10] and De Moor[11], respectively – referred to the alien population in Swaziland and not South Africa.

Freshwater crayfish have been documented as carriers of several pathogens including viruses, bacteria, fungi, and protozoan and metazoan parasites.[12-14] One of the metazoan parasites associated mostly with freshwater crayfish, and of particular interest here, is the temnocephalans (Platyhelminthes, Temnocephalida).[15] As well as parasitising crayfish, temnocephalans have been reported to also parasitise freshwater crabs and shrimps[16], molluscs and turtles[17] and isopods[18]. Records exist for two species of temnocephalans in South Africa. Mitchell and Kok[19] reported *Temnocephala chaeropsis* Hett, 1925 on *C. tenuimanus* imported from Australia, at a farm in the Free State Province, and, later, Avenant-Oldewage[20] found the same temnocephalan on the same host at a fish farm in the Gauteng Province. This worm is now known to be *Temnocephala minor* Haswell, 1888. In 1999, examination of the crayfish *C. destructor* from the Gariep Dam Fisheries Station in the Free State revealed another temnocephalan infection. In this case the temnocephalan was identified as *Diceratocephala boschmai* Bear, 1953 (Van As JG 2000, written communication, October 16). To date no record exists of natural populations of any temnocephalans in Africa.

Our aim here is to report on the first record of *C. quadricarinatus* and its temnocephalan ectoparasite established in the wild in South Africa.

## Materials and methods

As part of a larger study on the biodiversity of fishes, amphibians, aquatic reptiles and aquatic invertebrates in the lower Phongolo River, samples were collected from the outlet of Lake Nyamiti in the Ndumu Game Reserve during November 2012. The study was approved by the North-West University (approval no. NWU-00095-12-A4). Collection techniques included cast netting for fishes and baited crayfish traps for terrapins. Both these techniques resulted unexpectedly in the collection of an alien freshwater crayfish. The captured crayfish were maintained in aerated river water in a 20-L plastic container for later identification and closer examination for potential ectoparasites.

Examination of crayfish under a Nikon SMZ445 dissecting microscope (IMP Scientific & Precision, Boksburg, South Africa) revealed the presence of various life stages of an ectoparasitic temnocephalan flatworm. The temnocephalans were removed using a small camel hair brush and transferred to a glass Petri dish containing river water. Photographs of live temnocephalan adults were taken using a pocket camera through the eyepiece tube of the dissecting microscope and photographs of eggs on the host were photographed using a Nikon AZ100M microscope fitted with a digital camera. Two temnocephalans were later fixed under coverslip pressure in 10% neutral buffered formalin and three were fixed in 70% ethanol. Formalin-fixed specimens were subsequently hydrated, stained in alum carmine, dehydrated and mounted in Canada balsam. Fixed and stained specimens were examined using a Nikon E800 compound microscope and measured using Nikon NIS Elements software.

## Results

A total of four freshwater crayfish were collected above and below the low water bridged at the outlet of Lake Nyamiti (also referred to as Nyamiti Pan) in the Ndumu Game Reserve (26.882775 S, 32.311393 E) (Figure 1). All four specimens of crayfish examined were identified with the aid of Austin & Knott[21] as *C. quadricarinatus* (Figure 2) based on: an elongated chela; a long, sharp rostrum with raised rostral carinae; a rostrum with a long sharp apex spine and three or more long sharp marginal spines; and, lastly, raised and prominent postorbital ridges with a sharp spine at the anterior end. The largest of the four crayfish had hundreds of its larvae attached to the pleopods. One crayfish was infected with five temnocephalan specimens (Figure 3a). Based on the temnocephalan's almost complete covering of cilia, only two tentacles, a single pair of testes and the lack of a posterior attachment organ, it was identified as *Diceratocephala boschmai* (see Jones & Lester[22]). This material is similar to the type specimens of *D. boschmai* collected in New Guinea[23] as well as to populations found later in northern Australia.[15,22] Numerous fresh and embryonated temnocephalan eggs, as well as empty egg capsules (Figure 3b), were observed between the walking legs, other parts of the carapace and abdomen, on the hinges between the podomeres of the legs and in the head region. Empty egg cases remained attached to the crayfish. Judging by the effort required to remove eggs as well as empty egg cases manually, it appears likely that they remain permanently attached and are only discarded at the next moult.

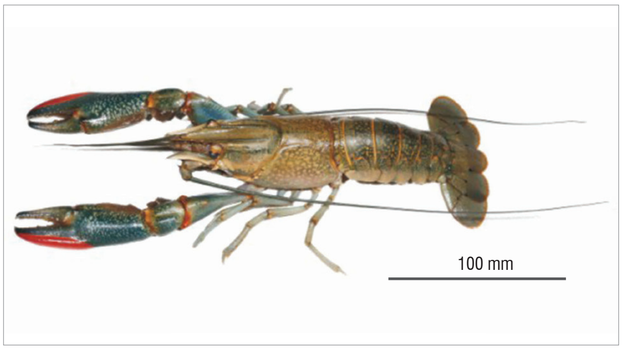
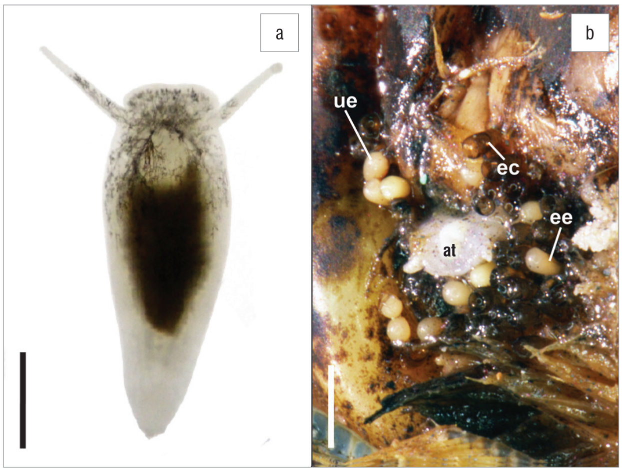



**Figure 2:** A male *Cherax quadricarinatus* specimen collected in the Ndumu Game Reserve, KwaZulu-Natal.




*Scale bar = 1 mm*

*ec, egg capsule; ee, embryonated egg; ue, unembryonated egg; at, adult temnocephalan*

**Figure 3:** Images of (a) a live *Diceratocephala boschmai* and (b) an adult specimen, eggs and empty egg capsules of *D. boschmai* on the ventral surface of *Cherax quadricarinatus.*

## Discussion

De Moor[11] summarised the potential impact of *C. quadricarinatus* as the destruction of living aquatic macrophytes, resulting in effects on ecosystem functioning, disturbance of breeding of bottom-spawning fish, introduction of undesirable associated organisms, such as parasites, and impacts on benthic macro-invertebrate communities. De Moor[11] further stated that the warmer regions of South Africa are likely to be more susceptible to the negative impacts of an invasion and pointed specifically to an infestation in Maputaland (thus including the Ndumu Game Reserve). Using the unified invasion framework as proposed by Blackburn et al.[24], this crayfish and its symbionts' invasion is already in at least a C3 category: 'Individuals surviving in the wild in location where introduced, reproduction occurring, and population self-sustaining'. However, because it is highly unlikely that the point of introduction was the Ndumu Game Reserve, it is possible that this invasion might already be in a D2 category: 'Self-sustaining population in the wild, with individuals surviving and reproducing a significant distance from the original point of introduction'. Resolving this extent must be a priority for future studies.

As the outlet of Lake Nyamiti is above the confluence of the Phongolo and Usutu Rivers, the potential for *C. quadricarinatus* to spread and colonise the Phongolo floodplains is of great concern. Similarly, the potential of this alien crayfish to establish downstream in Mozambique cannot be excluded. Both possibilities merit further investigation.

According to Jones and Lester[25], *D. boschmai* is not host specific and such temnocephalans do not successfully infest freshwater shrimps as they are removed by the shrimps' well-developed grooming behaviour. However, in freshwater crayfish, grooming behaviour is less well developed and, according to Jones and Lester[25], insufficient to prevent temnocephalans from infesting these hosts successfully. These authors are also of the opinion that the grooming ability of potential crustacean hosts is the only factor controlling host-specificity of *D. boschmai*. Brachyuran crabs do not display any grooming behaviour[26] and may therefore be highly susceptible to infestation if exposed to populations of *D. boschmai.* Oki et al.[27] reported that temnocephalans in Japan have the ability to survive on freshwater crabs. Avenant-Oldewage[20] conducted experiments that revealed *Temnocephala chaeropsis* can spread to the South African crab *Potamonautes warreni*.

There are no studies on the effect of temnocephalans on hosts other than freshwater crayfish. Given that *C. quadricarinatus* is present in the rivers of northern KwaZulu-Natal, we suggest that studies be undertaken to determine the extent of the invasion and whether *D. boschmai* has spread to other crustaceans, especially indigenous crabs, in the ecosystem. The effect that these temnocephalans could have on native freshwater crabs or on the aquatic systems in southern Africa is unknown and difficult to predict accurately. There are, however, several examples of alien aquatic species becoming invasive and destructive in South Africa's aquatic systems.[28]

The discovery of *C. quadricarinatus* infested with *D. boschmai* in the waterways of northern KwaZulu-Natal can thus be viewed as a double blow to the conservation of South Africa's freshwater ecosystems. This is of particular significance as not only were these aliens present in a protected area, but both were in breeding condition and had produced offspring (a C3, or possibly D2, stage of invasion[24]). This discovery further emphasises the importance of regulating exports and imports of potentially invasive aquatic fauna and flora – not only within the southern African subregion, but globally.

## Acknowledgements

We thank Dr Rob McCormac, Research and Aquaculture Director of Australian Aquatic Biological P/L for confirming the identification of the crayfish; Dr Kim Sewell, The University of Queensland, for confirming the identity of the temnocephalan; Prof. Angela Russell, Kingston University, UK for proofreading the manuscript; Ezemvelo KZN Wildlife for research permits OP 674/2012, OP 4709/2012, OP 4711/2012 and OP 4713/2012; the Water Research Commission for research funding (Project K5-2185); and all the members of the Phongolo floodplain research team for assistance in the field.

## Authors' contributions

Both authors contributed equally to the project design, collection of data, data analyses and writing of the manuscript.

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