Journal of Helminthology (2008) **82**, 365–371 doi:10.1017/S0022149X08054126

Nematodes from Swainson's spurfowl *Pternistis swainsonii* and an Orange River francolin *Scleroptila levaillantoides* in Free State Province, South Africa, with a description of *Tetrameres swainsonii* n. sp. (Nematoda: Tetrameridae)

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Abstract

Five Swainson's spurfowl collected in Free State Province, South Africa, were examined for helminth parasites, and the nematodes *Acuaria gruveli*, *Cyrnea parroti*, *Gongylonema congolense*, *Subulura dentigera*, *Subulura suctoria* and a new *Tetrameres* species were recovered. Their respective prevalence was 100, 20, 80, 20, 20 and 20%. These nematodes are all new parasite records for Swainson's spurfowl, and *Acuaria gruveli* constitutes a new geographical record as well. A single specimen of *Cyrnea eurycerca* was found in an Orange River francolin, representing a new host and geographical record for this parasite. The new species, for which the name *Tetrameres swainsonii* is proposed, can be differentiated from its congeners by a combination of the following characters of males: two rows of body spines, a single spicule which is 1152–1392 μm long, and eight pairs of caudal spines arranged in two ventral and two lateral rows of four spines each. The single female has the globular shape typical of the genus.

Introduction

Swainson's spurfowl *Pternistis swainsonii* (Smith, 1836) (Phasianidae: spurfowls) is endemic to southern Africa. In South Africa it has undergone a major southward range expansion and can now be found east of approximately 23°E and south as far as 30°S in the Eastern Cape, Free State, Gauteng, Limpopo, Mpumalanga, Northern Cape and North West Provinces. It is absent from the coastal lowlands of KwaZulu-Natal Province (Little, 2005). Its preferred habitat in South Africa is dense grassland in proximity to cultivated lands, where it exploits crops and associated insects. While some authors refer to Swainson's spurfowl as one of the most water-dependent perdicine birds in Africa (del Hoyo *et al.*, 1994; Little, 2005), a study in Limpopo Province, South Africa, revealed no or little reliance on easily accessible drinking water and birds seldom drank (Jansen & Crowe, 2002).

The Orange River francolin *Scleroptila levaillantoides* (Smith, 1836) (Phasianidae: francolins) is found in two distinct geographical areas on the African continent

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(del Hoyo *et al.*, 1994). While it is a frequent to common bird in Ethiopia and Somalia, numbers appear to have declined in its southern population, especially in South Africa and Namibia. This is thought to be mainly due to habitat pressure following the conversion of natural grass- and woodland habitats into farmland, despite the fact that, like Swainson's spurfowl, it will forage at the edges of cultivated land (Little *et al.*, 2000). The natural range of Orange River francolin in South Africa used to be restricted to north-western Northern Cape Province (del Hoyo *et al.*, 1994), but it has expanded to include north-eastern Eastern Cape Province, and Free State and North West Provinces, as well as the region east of the highveld of Mpumalanga and Gauteng Provinces (Little *et al.*, 2000; Little, 2005).

Only incidental findings on helminth parasites of both these gamebirds in South Africa have been published. Oosthuizen & Markus (1967) collected *Subulura* sp. from a single Swainson's spurfowl, while the only record pertaining to helminths of *S. levaillantoides* is that of Bennett *et al.* (1992) who reported *Microfilaria* sp. when cataloguing haematozoa of sub-Saharan birds.

This paper reports on helminths collected from the gastrointestinal tract (GIT) of five Swainson's spurfowl and a single Orange River francolin in Free State Province, South Africa and describes a new nematode, *Tetrameres swainsonii*, from the proventriculus of the former.

Materials and methods

Five Swainson's spurfowl, a single second-year male and four adult females (at least third-year), and a single adult male Orange River francolin were collected during a gamebird hunt in the vicinity of Petrus Steyn (27°39′S; 28°8′E), Free State Province, in August 2007. The habitat in the survey area was made up primarily of cereal plantings (maize) and sunflower, in a mosaic of grazing land.

Within 4 hours of being shot, the entire GIT was removed from the birds and placed in a plastic tray. The crop was ligated at the entrance of the oesophagus and the entrance to the proventriculus. The proventriculus was separated from the gizzard, and the small intestine was separated from the gizzard and caeca. The GITs of the various birds were placed in individual containers, stored at 2°C overnight and then fixed in 70% ethanol.

Subsequently, the crop, proventriculus, gizzard, small intestine and caeca were washed separately over a 150 μm sieve and, together with the residue, examined under a stereoscopic microscope. Helminths in the gizzard usually only became visible after removal of the lining.

All helminths were stored in 70% ethanol. For identification purposes, nematodes were cleared in lactophenol and studied under a standard microscope. Intensity of infection, mean intensity of infection, mean abundance and prevalence are used in accordance with Margolis *et al.* (1982).

Results

All five Swainson's spurfowl harboured nematodes and a total of six species, *Acuaria gruveli* (Gendre, 1913), *Cyrnea parroti* Seurat, 1917, *Gongylonema congolense* Fain, 1955, *Subulura dentigera* Ortlepp, 1937, *S. suctoria* (Molin, 1860) and *T. swainsonii* n. sp., was recovered. Their habitat, prevalence, mean intensity of infection and mean abundance are listed in table 1. A single host harboured a total of four species, a second three, and three birds had two nematode species each. The mean species richness was 2.6 (SD = 0.9). The intensity of infection ranged from 3 to 68, with a mean intensity of 19 (SD = 27.7). The second-year male had the highest species diversity as well as highest intensity of infection.

Two nematode species were recovered from both the gizzard and caeca, and a single nematode species from the proventriculus and crop, respectively. No helminths were found in the small intestine.

With the exception of a single *C. eurycerca* Seurat, 1914 in its gizzard, the Orange River francolin harboured no helminth parasites.

The presence of *A. gruveli* in Swainson's spurfowl constitutes both a new host record and a new geographical record for this parasite, while *C. parroti*, *G. congolense* and *S. suctoria* are new parasite records for this host. This is the first report of *S. dentigera* from a host other than helmeted guineafowl *Numida meleagris* (Linnaeus, 1758) (Phasianidae: guineafowls). *Cyrnea eurycerca* is recorded from Orange River francolin as well as from South Africa for the first time.

Tetrameres swainsonii n. sp

Description. *Tetrameres swainsonii* is described from four males and one female from a single Swainson's spurfowl. Males were found free in the lumen of the proventriculus, while the female was dissected from the proventricular glands. All measurements are in micrometres unless otherwise stated (fig. 1).

Female. Bright red *in situ* as typical for the genus, damaged; only buccal capsule, 24 deep and 16 wide, maximum body width (3 mm) and length (4 mm) as

Table 1. Nematodes recovered from five Swainson's spurfowl *Pternistis swainsonii* in Free State Province, South Africa.

Nematode	Habitat	Prevalence (%)	Mean intensity (± SD)	Range	Mean abundance (± SD)
Acuaria gruveli	Gizzard	100	2.4 (0.9)	1–3	2.4 (0.8)
Cyrnea parroti	Gizzard	20	2.0	2	0.4 (0.8)
Gongylonema congolense	Crop	80	4.25 (5.9)	1–13	3.4 (4.8)
Subulura dentigera	Caeca	20	12.0	12	2.4 (4.8)
Subulura suctoria	Caeca	20	47.0	47	9.4 (18.8)
Tetrameres swainsonii n. sp.	Proventriculus	20	5.0	5	1.0 (2.0)

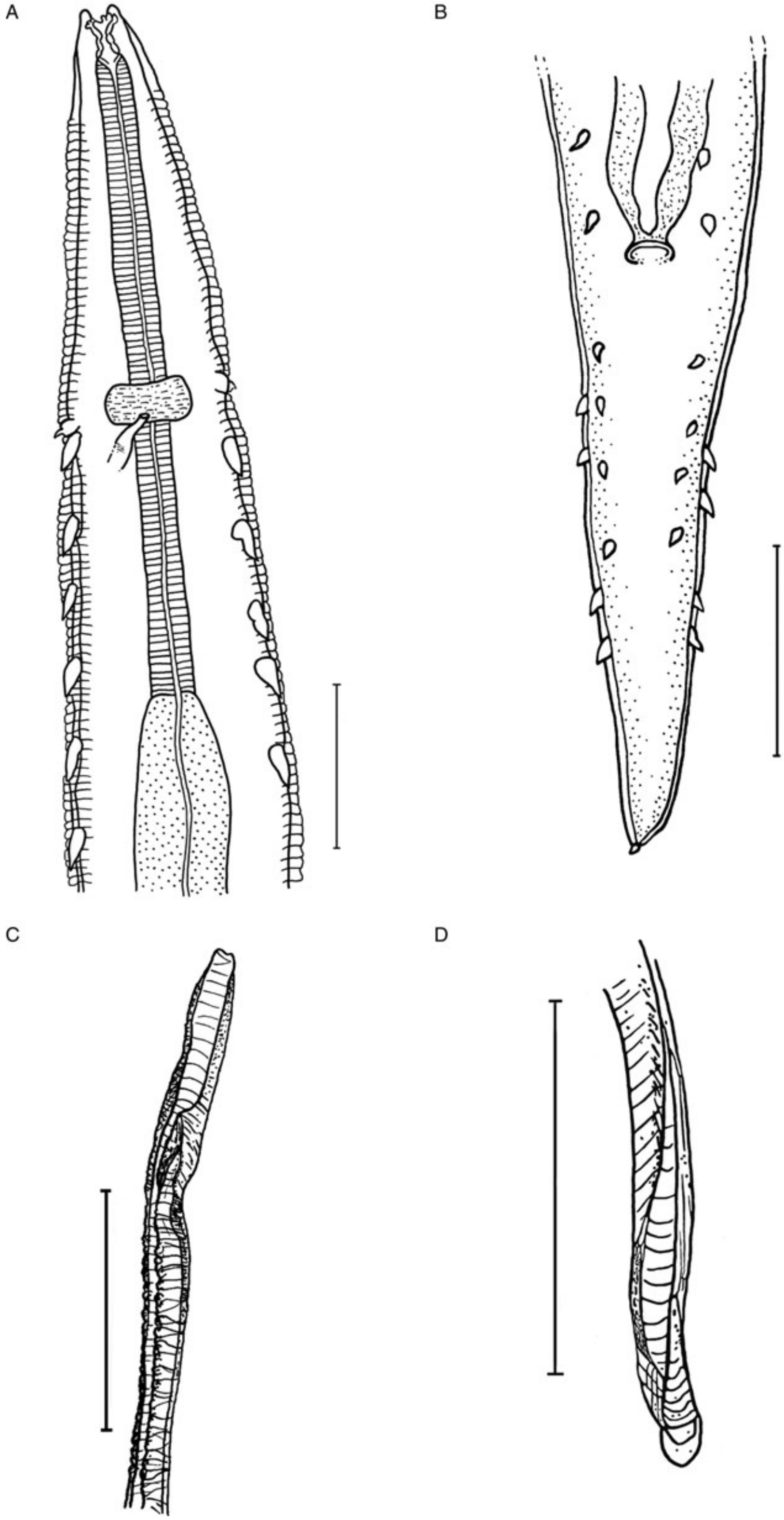

Fig. 1. *Tetrameres swainsonii* n. sp. male. (A) Ventral view of anterior extremity illustrating the position of the deirids, nerve ring, excretory pore and first pair of body spines. (B) Ventral view of posterior extremity showing the arrangement of the caudal spines. (C) Proximal end of the single spicule, lateral view. (D) Distal end of the spicule, lateral view. Scale bars = 100 μm.

well as egg length and width could be measured. Eggs ($n = 10$), length 49 (SD = 2.98), between 43 and 52, width 32 (SD = 1.47), between 30 and 34; polar filaments not seen. Body globular with anterior and posterior extremities forming short protuberances; surface divided into four segments by four conspicuous longitudinal cuticular grooves; each segment with numerous transverse striations.

Male. Measurements of holotype male given in text, those of two paratypes and a further specimen in table 2. Body elongated, tapered at both ends, 5.1 mm long and 188 wide. Cuticle striated transversely as well as longitudinally. Cuticular spines arranged in two lateral rows, one dorsal and one ventral to inconspicuous lateral alae; 41 spines per row in holotype, 40 to 43 in paratypes; first pair of spines at 269 and 285 from anterior extremity. Deirids with apical spines at 261 and 251 from anterior extremity. Nerve ring and excretory pore at 252 and 265 from apex, respectively. Deirids at approximately centre of nerve ring with first pair of cuticular spines in close proximity, but posterior to deirids. Excretory pore in same vicinity, sometimes slightly anterior, slightly posterior or on same level as first pair of cuticular spines (fig. 1A). Depth of buccal capsule 19, inner diameter 6. Oesophagus divided into muscular and glandular parts, 412 and 914, respectively; total length of oesophagus 1326. Single spicule, slender, 1384 long, trough-shaped with spatulate, almost square tip (fig. 1D); proximal tip slightly angled away from longitudinal axis (fig. 1C). Gubernaculum absent. Tail 330 long, with short pointed tip. Eight pairs of caudal spines arranged in two ventral and two lateral rows, containing four spines each (fig. 1B).

Specific diagnosis. *Tetrameres swainsonii* n. sp. is characterized by two rows of body spines, starting just posterior to the deirids situated at the level of the nerve ring. The single spicule is 1152 to 1392 long, and 16 caudal spines are arranged in two ventral and two lateral rows, each bearing four spines.

Host. Swainson's spurfowl *Pternistis swainsonii* (Smith, 1836).

Habitat. Males occur free in the lumen of the proventriculus, females are sedentary in proventricular glands.

Locality. Vicinity of Petrus Steyn (27°39′S; 28°8′E), Free State Province, South Africa.

Etymology. The specific epithet *swainsonii* refers to the host.

Deposition of type specimens. Holotype male: 2008.6.20.1, allotype female, paratype males: 2008.6. 20.2–5.

Taxonomy of Tetrameres

To date three species belonging to the genus *Tetrameres* have been described from avian hosts in South Africa, *Tetrameres paradisea* Ortlepp, 1932 from Stanley's crane *Anthropoides paradiseus* (Lichtenstein, 1793) (Gruidae: cranes), *Tetrameres prozeskyi* (Ortlepp, 1964) from red-billed and southern yellow-billed hornbills *Tockus erythrorhynchus* (Temminck, 1823) (Bucerotidae: typical hornbills) and *Tockus leucomelas* (Lichtenstein, 1842) (Bucerotidae: typical hornbills), respectively, and *Tetrameres numida* Junker & Boomker, 2007 from helmeted guineafowl. *Tetrameres paradisea* is similar to the new taxon in that it has two rows of cuticular spines and possesses a single spicule. However, Ortlepp (1932) illustrates three cuticular spines anterior to the deirids, with the latter placed well anterior to the nerve ring, whereas in the present specimens, the first pair of cuticular spines only appears posterior to the deirids, and both the first pair of cuticular spines and the deirids are in the immediate vicinity of the nerve ring. Moreover, the spicule length of *T. paradisea* only reaches 690 as opposed to a minimum length of 1152 in the present specimens.

In *T. prozeskyi* a single spicule measuring 230–260 is usually present and in those instances where a second spicule was found, it was shorter than the first (Ortlepp, 1964). A further distinguishing feature between *T. prozeskyi* and *T. swainsonii* n. sp. is the presence of four rows of cuticular spines in the former (Ortlepp, 1964) versus two rows in the latter. Only 12 caudal spines were reported for *T. prozeskyi* as well as for *T. paradisea* (Ortlepp, 1932, 1964) as opposed to the 16 caudal spines seen in the new taxon.

Like *T. swainsonii* n. sp., *T. numida* is characterized by two rows of cuticular spines, but the arrangement of the first pair of spines, the deirids and the nerve ring is

Table 2. Morphological characteristics of *Tetrameres swainsonii* n. sp. males from Swainson's spurfowl *Pternistis swainsonii*. All measurements in micrometres unless otherwise indicated.

Morphological criteria	Specimen A	Paratype 1	Paratype 2
Body length (mm)	4.7	4.8	5.1
Body width max.	203	200	216
Distance from apex to first pair of somatic spines	276; 260	250; 272	340; 340
Distance from apex to nerve ring	244	245	263
Distance from apex to deirids	243; 235	237; 242	268; 286
Distance from apex to excretory pore	282	275	310
Depth of buccal capsule	21	23	23
Width of buccal capsule (inner)	5	6	5
Muscular oesophagus	368	418	428
Glandular oesophagus	1005	914	1031
Oesophagus total length	1377	1285	1451
Length of tail	291	306	309
Length of single spicule	1152	1392	1183

distinctly different from that seen in the present specimens (Junker & Boomker, 2007a). The first pair of cuticular spines of *T. numida* is situated anterior to the deirids, which are approximately at the level of the second pair of cuticular spines, and the nerve ring is distinctly posterior to the deirids. Only 12 caudal spines are described for *T. numida* and, although additional ventral spines may occasionally be present, the two lateral rows consistently carried three spines each. In addition, *T. numida* possesses a right and a left spicule, ranging from 106 to 170 and from 1699 to 2304, respectively (Junker & Boomker, 2007a).

Of the 54 species of *Tetrameres* listed by Junker & Boomker (2007a), only *T. paradisea*, *Tetrameres grusi* Shumakovitsh, 1946, *Tetrameres pattersoni* Cram, 1933 and *Tetrameres puchovi* Gushanskaja, 1949 share the combination of two rows of cuticular spines and a single spicule with the present specimens. However, the spicules of *T. grusi* (638–783) and of *T. puchovi* (307–309) (Skrjabin & Sobolev, 1963) are distinctly shorter than those of *T. swainsonii* n. sp. (1152–1392). Moreover, the caudal spines of *T. grusi* are arranged in several irregular rows, and several pairs of cuticular spines originate anterior to the nerve ring (Skrjabin & Sobolev, 1963), whereas in *T. swainsonii* n. sp. the first pair of cuticular spines emerges posterior to the nerve ring. The distance from the apex to the deirids is 160 in *T. puchovi* (Skrjabin & Sobolev, 1963), which is considerably shorter than that observed in the new taxon, namely 235–286.

Tetrameres pattersoni is closest to *T. swainsonii* n. sp. in spicule length, with a single, strongly chitinized spicule of length 1200–1500; but it differs in the arrangement of caudal spines in three lateral and four subventral pairs, as opposed to four pairs each in the new taxon. The distance of the deirids from the apex, which is less than half that seen in *T. swainsonii* n. sp., namely 83–112 (Skrjabin & Sobolev, 1963), clearly separates *T. pattersoni* from *T. swainsonii* n. sp.

Discussion

The single second-year male Swainson's spurfowl yielded the largest number of helminth species as well as individuals. Phasianid chicks are reported to rely heavily on insect food in the early stages of their lives (del Hoyo *et al.*, 1994). Chicks of grey partridge *Perdix perdix* Linnaeus, 1758 (Phasianidae: partridges) in Europe, for example, consume a diet consisting of 80% insect matter for the first 2 weeks after hatching (del Hoyo *et al.*, 1994). Arthropods only make up approximately 7% of the crop weight of adult *P. swainsonii*, reaching a maximum of up to 20% in summer (del Hoyo *et al.*, 1994). Higher intake of live food by juvenile versus adult birds is likely to increase exposure to infected intermediate hosts, which, in turn, would result in higher worm burdens. However, because of the small sample size it is not possible to establish whether our findings are due to chance or reflect a true pattern in the helminth community of Swainson's spurfowl.

Only nematodes were collected from Swainson's spurfowl and the single Orange River francolin. This is noteworthy, especially taking into account that all nematodes collected from these two hosts are heteroxenous; that is, their life cycles include various arthropod intermediate hosts, such as orthopterans and coleopterans (Anderson, 1992), which in addition serve as intermediate hosts for cestodes and acanthocephalans (Moore, 1962; Reid, 1962). Moreover, helmeted guineafowl collected at the same locality during the course of this study harboured nematodes and cestodes as well as acanthocephalans (Davies *et al.*, in review), thereby confirming their presence in the environment.

While Swainson's spurfowl had a markedly less diverse helminth fauna than helmeted guineafowl at the study site, the former seem to be more suitable hosts of the gizzard nematode *A. gruveli*, since it was collected from all five spurfowl, but was absent in more than 40 helmeted guineafowl (Davies *et al.*, in review). Other galliform birds recorded as final hosts of *A. gruveli* include double-spurred spurfowl *Pternistis bicalcaratus* (Linnaeus, 1766) (= *Francolinus bicalcaratus*) (Phasianidae: spurfowls) in Togo (Quentin & Seureau, 1983), common quail *Coturnix coturnix* (Linnaeus, 1758) (Phasianidae: quails) in the Palearctic region (Baruš & Sonin, 1983) and red-legged partridge *Alectoris rufa* (Linnaeus, 1758) (Phasianidae: partridges) in Spain (Tarazona *et al.*, 1979), suggesting that perdicine birds feature more prominently in the life cycle of this parasite than do guineafowls.

A possible explanation for the presence/absence of helminths in Swainson's spurfowl versus helmeted guineafowl at the same locality might be a difference in their dietary preferences, which in turn would influence the probability of exposure to intermediate hosts of certain parasites. Moreover, differences in the immune competence of the two bird species might result in a higher resistance in guineafowl. Similarly, morphological differences between hosts, such as the nature of the gizzard lining, could prevent establishment of, for example, *A. gruveli* in guineafowl, but allow colonization of spurfowl.

Cyrnea parroti, *G. congolense* and *S. suctoria* collected from Swainson's spurfowl are also commonly found in other galliform birds (Junker & Boomker, 2007b). Contrary to this, *S. dentigera* had hitherto been recorded from helmeted guineafowl only.

Cyrnea eurycerca, which was present in the single Orange River francolin, seems a relatively common parasite in francolins and spurfowls, and has previously been collected from black francolin *Francolinus francolinus* (Linnaeus, 1766) (Phasianidae: francolins) in Italy, grey francolin *Francolinus pondicerianus* (Gmelin, 1789) (Phasianidae: francolins) in India and double-spurred spurfowl in Togo (Marconcini & Triantafillu, 1970; Jehan, 1974; Seureau & Quentin, 1983).

The low prevalence and intensity of infection of *T. swainsonii* n. sp. in Swainson's spurfowl is in keeping with data obtained for *T. numida* from helmeted guineafowls in Limpopo Province, as well as in the present study area (Junker & Boomker, 2007a; Davies *et al.*, in review). Similarly, only two of 158 bobwhite quail *Colinus virginianus* (Linnaeus, 1758) (Phasianidae: quails) examined in northern Florida harboured *T. pattersoni*, and intensity of infection ranged from 0 to 1 (Moore & Simberloff, 1990).

The overall low helminth diversity and intensity of infection seen in Swainson's spurfowl at the study site might be attributable to several factors. First, they occur in pairs or small family groups rather than in large flocks (Little, 2005; Jansen & Crowe, 2006), which would facilitate parasite transmission (Moore *et al.*, 1988). Jansen & Crowe (2002) reported a covey size ranging from 1 to 4. Second, the birds were collected in winter, when the volume of their diet consists mainly of grass seeds, weed seeds and agricultural seeds, while invertebrates play a minor role (Jansen & Crowe, 2006). In terms of crop volume, 5.74% is made up of invertebrates during the summer months and 3.64% during the winter months (Jansen & Crowe, 2006). Third, much of their habitat consisted of cultivated lands, the insect fauna of which might be depauperate because of low habitat diversity and the use of pesticides. In addition, while Swainson's spurfowl from a cereal-crop habitat, similar to that found in the current study area, ingested the greatest number and volume of invertebrates, when compared to savanna and a cotton habitat, more than 90% of the total number of invertebrates consumed consisted of lepidopteran larvae (Jansen & Crowe, 2006). The latter, however, have not been reported as intermediate hosts for nematode species recovered from Swainson's spurfowl and would thus have no influence on helminth diversity or intensity of infection in these birds.

Acknowledgements

We thank Mr J.P. Wales for access to 'The Jimmy Wales Shoot' for collection of gastrointestinal tracts. The research was supported financially by the Centre of Excellence in Birds at the Percy FitzPatrick Institute (funded by the South African Department of Science and Technology and the National Research Foundation), the University of Cape Town's Research Committee, and the Department of Veterinary Tropical Diseases, University of Pretoria. K.J. was supported by a University of Pretoria Postdoctoral Fellowship Grant.

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(Accepted 30 June 2008)
First Published Online 28 August 2008