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South African cycads at risk: *Aulacaspis yasumatsui* (Hemiptera: Coccoidea: Diaspididae) in South Africa

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The scale insect *Aulacaspis yasumatsui* is native to Southeast Asia and a major pest of cycad (Cycadales) plants. Due to an increase in worldwide trading of cycads, *A. yasumatsui* has spread globally and has become a major threat to many cultivated and native cycads worldwide. In this study we report formally, for the first time, *A. yasumatsui* infesting cycads in South Africa. This scale insect was observed infesting cycads in three provinces in South Africa, namely, Gauteng, KwaZulu-Natal and Limpopo. Its identification was based on morphology and nucleotide sequences of three gene regions. Although more common and damaging on non-native *Cycas* species, its presence on some native South African *Encephalartos* species is of concern and effort should be made to control the spread and impact of this pest in the country.

Key words: cycad aulacaspis scale, CAS, *Cycas*, *Encephalartos*.

INTRODUCTION

Cycads (Cycadales) are a group of seed plants originally thought to have appeared around 250 to 325 million years ago, making them the oldest living seed plants (Brenner *et al*. 2003). A recent study, however, has shown that cycads have gone through a second wave of evolution and it is more than likely that extant cycads evolved around 12 million years ago (Nagalingum *et al*. 2011). Many cycad species, including some cycad genera, are facing mass extinction in the wild, mainly due to human activities such as illegal trading and habitat destruction (IUCN/SSC-CSG 2005). Additionally, biotic factors, such as insect pests and microbial pathogens that affect these plants, are on the increase.

Armoured scale insects (Diaspididae) are known as major pests of mature leaves and trunks of many cycad species (Grobbelaar 2004). The Diaspididae is the largest scale insect family of the superfamily Coccoidea within the order Hemiptera, with over 2400 described species (Miller & Davidson 2005). They are among the most invasive insects in the world, colonizing all continents except Antarctica (Andersen *et al*. 2010). The Diaspididae occur on a wide range of plant hosts, which encompass more than 1380 plant genera (Miller 2005). In addition to their wide host range, armoured scale insects are the most polyphagous insects known, with some species feeding on more than 100 plant species (Miller & Gimpel 2009). Due to their invasive ability as a result of their small size, waxy protective layer and difficulty of identification, the Diaspididae are a major quarantine threat (Burger & Ulenberg 1990).

The scale insect *Aulacaspis yasumatsui* Takagi (Hemiptera: Diaspididae), commonly known as the cycad aulacaspis scale (CAS), has recently become a major threat to many cultivated and native cycads globally (Song *et al*. 2012). CAS was originally described in 1977 from specimens collected by K. Yasumatsui in 1972 and 1973 from a *Cycas* species in Bangkok, Thailand (Takagi 1977). This scale insect infests plants and reduces their longevity by populating leaves, fruits, trunks and sometimes roots of the plants, resulting in chlorosis and premature death of leaves (Cave 2006). CAS is native to Southeast Asia where its numbers are kept at low levels by natural predators (Tang *et al*. 1997).

CAS is referred to as the 'single most important threat to natural cycad populations' by the International Union for Conservation of Nature/Species Survival Commission-Cycad Specialist Group (IUCN/SSC-CSG), a subgroup on invasive pests, formed in 2005. They state that two species of cycads face 'imminent extinction' in the wild as a

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result of CAS. The IUCN/SSC-CSG has, therefore, given CAS top priority status. CAS is currently listed in terms of the South African 'Alien and Invasive species Regulations' as prohibited in South Africa (National Environmental Management: Biodiversity Act 10 of 2004; Department of Environmental Affairs 2014).

Due to an increase in cycad trading, CAS has spread to many parts of the world, including the Cayman Islands, China, Guam, Hong Kong, Singapore, Taiwan, the Hawaiian islands, Puerto Rico, U.S. Virgin Islands and Vieques Island (CABI/EPPO 2000; IUCN/SSC 2005). The first report of CAS on cycads outside Thailand was in 1996 from Florida, U.S.A., where it was observed infesting ornamental plants and killing large numbers of king sago, *Cycas revoluta* Thunberg (Cave *et al*. 2009). Since its first report in the U.S.A. it has become a major threat to native cycads globally, killing large numbers of *Cycas micronesica* Hill in Guam (Terry & Marler 2005) and *Cycas taitungensis* Shen, Hill & Tsou in Taiwan (IUCN/SSC 2005). The first report of CAS from Africa resulted from its detection on cut foliage of cycads imported to France from the Ivory Coast (Germain & Hodges 2007; ISSG 2011; https://gd.eppo.int/taxon/AULSYA/distribution).

Recently, two South African private collectors reported CAS infesting, and completely covering, certain *Encephalartos* species in their gardens (Craig & Paul 2011, http://www.cycadsg.org). It is, however, not clear if these identities were confirmed by an entomologist and according to the ScaleNet database (http://www.sel.barc.usda.gov/scalenet/scalenet.htm), CAS has yet to be reported from South Africa at that time. Also, until now, it had not been detected in this country despite collecting efforts by local entomologists searching for insects on cycads. CAS has, however, been reported to affect African cycad species grown as non-natives in the U.S.A. Based on these reports, *E. barteri* Miguel, *E. ferox* Bertol, *E. hildebrandtii* nr. *lebombensis* Braun & Bouch, *E. manikensis* Gilliland, *E. pterogonus* Dyer & Verd, *E. whitelockii* Hurter and also *Stangeria eriopus* Baill are susceptible to this insect (Weissling & Howard 1999).

Recently, leaf and cone samples of a *Cycas thouarsii* plant from the Durban Botanical Gardens in KwaZulu-Natal (KZN) were brought to our laboratories for the identification of the cause of an unsightly white crust on their surfaces. In this study we describe the identification of this phenomenon, caused by an armoured scale insect, based on morphology and DNA sequence data. We also report on subsequent findings of the same scale insect problem in other parts of South Africa.

MATERIALS AND METHODS

Insect specimens

Plant samples bearing white, scale-like insects were obtained from the Durban Botanical Gardens (KwaZulu-Natal Province), and subsequently from Pretoria (Gauteng Province), Phalaborwa and Tzaneen (Limpopo Province), as well as Kwambonambi and Warner Beach (KwaZulu-Natal Province), South Africa. Samples included cone sections, leaves and leaf bases. Specimens were transferred to 90 % ethanol for storage at –4 °C and further study. Reference voucher specimens have been deposited in the South African National Collection of Insects at the Agricultural Research Council in Pretoria (SANC) and the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria.

Identification: morphology

For morphological study, adult females were mounted on microscope slides to facilitate the detailed examination of minute dermal features under high magnification that is necessary for scale insect identification. Specimens were prepared and slide-mounted mainly according to the method described by Williams & Granara de Willink (1982), except that the de-waxing procedure in step three was omitted, the staining fluid used was mixed according to Cilliers (1967), and specimens were dehydrated in glacial acetic acid instead of absolute alcohol in step five. Morphological identification was done using published keys for CAS, and by comparison with descriptions and illustrations of this species (Hodges *et al*. 2003; Malumphy & Marquart 2012; Miller & Davidson 2005; Suh & Hodges 2007; Suh & Ji 2009; Takagi 1977; Takagi & De Faveri 2009).

Identification: DNA sequencing

Representative insect specimens from each geographic region were separated individually from their protective scales under a stereo microscope and stored in 100 % ethanol at –4 °C for 24 h. Prior to DNA extraction, samples were washed twice with sterile water to remove excess ethanol. Genomic DNA extraction was performed accord-

ing to the CTAB (cetyl trimethyl ammonium bromide) protocol described by Möller *et al*. (1992). Reagents used were scaled down to 10 % of each volume, in order to accommodate small sample sizes. The genomic DNA concentration was determined using a Thermo Scientific Nanodrop® 2000 Spectrophotometer (NanoDrop Technologies, Wilmington, DE, U.S.A.). PCR amplification of a DNA fragment spanning the D2 and D3 expansion segments of the 28S, the Translation Elongation factor (TEF) 1α and a region of mitochondrial DNA encompassing the 3′ portion of cytochrome oxidase I (COI), and the 5′ portion of cytochrome oxidase II (COII), was performed using reagents provided by KAPA Taq PCR Kit (Kapa Biosystems, Cape Town, South Africa). PCR protocols reported by Morse & Normark (2006) and Provencher *et al*. (2005) were used to amplify the 28S and EF1α, and COI-COII regions, respectively. In the PCR reactions, the 28S primer s3660 (GAGAGTTMAASAGTAC GTGAA-AC), from Morse & Normark (2006), was paired with the primer 28b (TCGGAAGGAACCA GCTACTA) from Whiting *et al*. (1997) to partially amplify the 28S genomic region. Primer EF-1α (GATGCTCCGGGACAYAGA) from Morse & Normark (2006) was paired with primer EF2 (ATGTGAGCGGTGTGGCAATCCAA) from Palumbi (1996) for the TEF region. Primer C1-J-2753ywr (GTAAACCTAACATTTTTYCCWCA RCA) from Provencher *et al*. (2005) was paired with primer C2-N-3662, from Simon *et al*. (1994), for the COI-COII region. All PCR reactions were performed with a positive control for accuracy and a negative control to avoid false positives as a result of contamination. PCR products were stained with GelRed™ (Biotium, U.S.A.) and visualized using a 1.5 % agarose gel. PCR products were cleaned using ExoSAP enzymatic digestion (Affymetrix, CA, U.S.A.).

Forward and reverse sequencing reactions were performed at a final volume of 12 μl with the same primers as used for the PCR amplification reactions. Sequencing reaction mixtures contained 2.5 μl sequencing buffer, 0.5 μl BigDye terminator cycle sequencing kit (Applied Biosystems, Foster City, CA, U.S.A.), 1 μl of the selected primers (10 mM) and 4 μl purified PCR product. The thermal cycling conditions comprised 25 cycles of 10 s at 96 °C, 5 s at 54 °C (TEF1α and 28S) or 52 °C (COI-COII) and 4 min at 60 °C. Sequencing PCR products were purified using ethanol precipitation of DNA. Sequencing was performed on an ABI PRISM™ 3100 DNA Analyzer (Applied BioSystems, Foster City, CA, U.S.A.).

Phylogenetic analyses

DNA sequences were edited using MEGA5.2 and BioEdit and aligned using the online interface of MAFFT version 7 (http://mafft.cbrc.jp/alignment/software/). Appropriate substitution models for the three data sets (28S, EF1α and COI-COII) were determined using the Akaike Information Criterion (AIC) in jModelTest 2.1.5 (http://darwin.uvigo.es). The selected models were TIM3+G (gamma shape parameter = 0.1430; Pinvar = 0), TIM1+I (Pinvar = 0.5680) and TIM1ef+G (gamma shape parameter = 0.3800; Pinvar = 0) for 28S, COI-COII and TEFα, respectively. A maximum likelihood (ML) analysis was performed in PAUP4.0b10 (Swofford 2000) using random sequence addition and tree bisection-recognition (TBR) branch swapping. Sequences used in the ML analysis were obtained from GenBank as published by Morse & Normark (2006) and Andersen *et al*. (2010). The scale insect species *Chionaspis pinifoliae* was used as the outgroup. The confidence levels of the ML phylogenies were estimated using a bootstrap method with 1000 replications.

RESULTS

Symptoms, hosts and distribution

The scale insect was observed mostly on the undersides of cycad leaves, on cones and leaf bases. It was especially abundant on older cycad cones. The insect was very common on *C. thouarsii,* occurring on this plant in high numbers and leading to leaf chlorosis and death (Fig. 1a, b). It formed crusts on heavily infested plant parts (Fig. 1c, d, f). On native *Encephalartos* species, inspected in the Durban Botanical Gardens (Table 1), the insect was most abundant on *E. transvenosus* (Fig. 1e), with very low numbers on other species, mostly occurring on leaf bases. Specimens examined included those from the Durban Botanical Gardens and two private gardens in KwaMbonambi and Warner Beach (KwaZulu-Natal Province) respectively, a private garden in Phalaborwa (Limpopo Province) and a commercial nursery in Pretoria (Gauteng Province).

Identification: morphology

Slide-mounted adult female specimens keyed

Table 1. List of cycad species from which the scale insect was observed and the regions where the cycad species occur. Host recorded from: (CN = commercial nursery; G = garden).

Cycad species	Region
Cycas thouarsii	Warner Beach (G), Durban Botanical Gardens, Kwambonambi (G)
Cycas revoluta	Pretoria (CN), Phalaborwa(G)
Encephalartos ferox	Warner Beach (G), Durban Botanical Gardens
E. lebomboensis	Warner Beach (G)
E. longifolius	Durban Botanical Gardens
E. natalensis	Durban Botanical Gardens
E. paucidentatus	Durban Botanical Gardens
E. transvenosus	Durban Botanical Gardens
E. villosus	Warner Beach (G)

out as *Aulacaspis yasumatsui* in the keys to various economically important Diaspididae published by Miller & Davidson (2005), Suh & Hodges (2007) and Suh & Ji (2009). Important distinguishing features observed in the material studied, and which confirmed its identity as CAS, were the distinctly rounded prosoma (Fig. 2a), an absence of dorsal macroducts on the first and second abdominal segments; fourth pygydial lobes not being well-developed, instead being represented by simple raised sclerotized areas; the absence of projecting lateral sclerotizations on either side of the labium; and the presence of dorsal microducts on the submedian areas of abdominal segments 1 and 2. The medial lobes of the pygidium were divergent and recessed into the pygidium to form a notch (Fig. 2b). Specimens examined also had a distinctive area of ornamentation on the ventral submedian area on either side of the abdomen at the distal edges of segments 2–5 (Fig. 2c). Dorsal median macroducts were present on the third and fourth abdominal segments, but absent from first two abdominal segments (Fig. 2d).

Phylogenetic analysis

Amplification reactions of the 28S, TEF1α and COI-COII gene regions produced fragments of approximately 700 base pairs (bp), 900 bp and 800 bp, respectively. Successful sequence reactions were obtained only for the Durban and Kwambonambi specimens. Blast searches of the resultant sequences in the GenBank database showed that the insects belonged to the genus *Aulacaspis* and were most closely related to *A. yasumatsui*. For all three data sets a 50 % majority rule bootstrap tree was obtained from a maximum likelihood analysis (Figs 3–5). In the TEF1α and COI-COII trees the South African specimens, represented by samples from the Durban Botanical Gardens (*HC* 7220) and Kwambonambi (*HC* 7226), grouped together with *A. yasumatsui*, supported with a bootstrap value of 100 % (Figs 3, 4). In the 28S tree the sequenced insects also grouped with *A. yasumatsui.* However, this grouping was not as well supported, with a bootstrap value of 79 % (Fig. 5). Minor sequence differences were found between South African specimens of *A. yasumatsui* and those in GenBank (2 and 4 % respectively); however, these are less than that observed between *A. yasumatsui* and other species of *Aulacaspis.* Percentage sequence differences between *A. yasumatsui* and *A. rosae*, its closest phylogenetic relative, for example, are 7 %.

DISCUSSION

This study represents, to our knowledge, the first confirmed report of the important quarantine pest *Aulacaspis yasumatsui* (CAS) in South Africa. Identification was based both on morphology and DNA sequence data. Previous to this study there had been two other reports of CAS in South Africa (Craig & Paul 2011). Those reports, however, do not appear on the ScaleNet database or any other official publication, and do not indicate whether specialist taxonomic assistance was sought to confirm that the scale insects were definitely CAS. Our study also provides evidence that CAS is not only present in South Africa, but is widespread, occurring in three provinces (Gauteng, KwaZulu-Natal and Limpopo) on native and non-native cultivated cycad species.

Aulacaspis is a genus of about 90 species that are

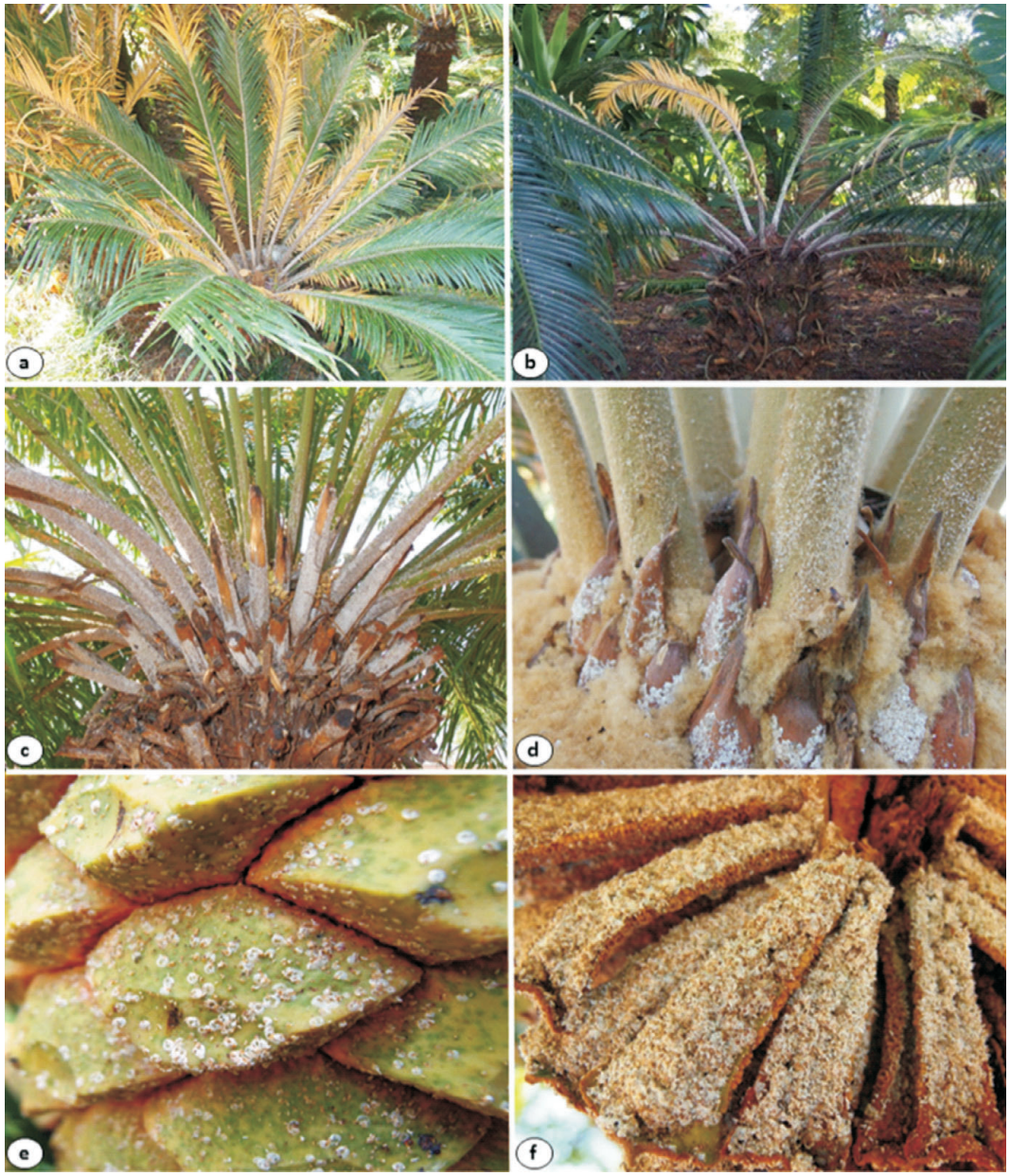

Fig. 1. **a** & **b**, Leaf chlorosis on *Cycas thouarsii* due to infestation by the scale insect. Scale insect colonization on *Cycas thouarsii* (**c**) leaf bases and (**d**) cataphylls, and on (**e**) *E. transvenosus* and (**f**) *C. thouarsii* cones.

found mostly in the eastern Palearctic and Oriental regions (Miller & Gimpel 2009). Members of the genus are generally characterized by their swollen prosoma, with the distended head and/or pro- and mesothorax being wider than the rest of their elongate bodies. The prosoma is often somewhat quadrate, with parallel sides. The medial lobes of the pygidium are usually divergent, recessed into the pygidium to form a notch, and have serrated inner edges. Another notable feature is the distinctive area of ornamentation or puckering of the derm in the form of a 'petal-like cluster', on the ventral submedian area on either side of the abdomen at the distal edges of segments 2–5. The

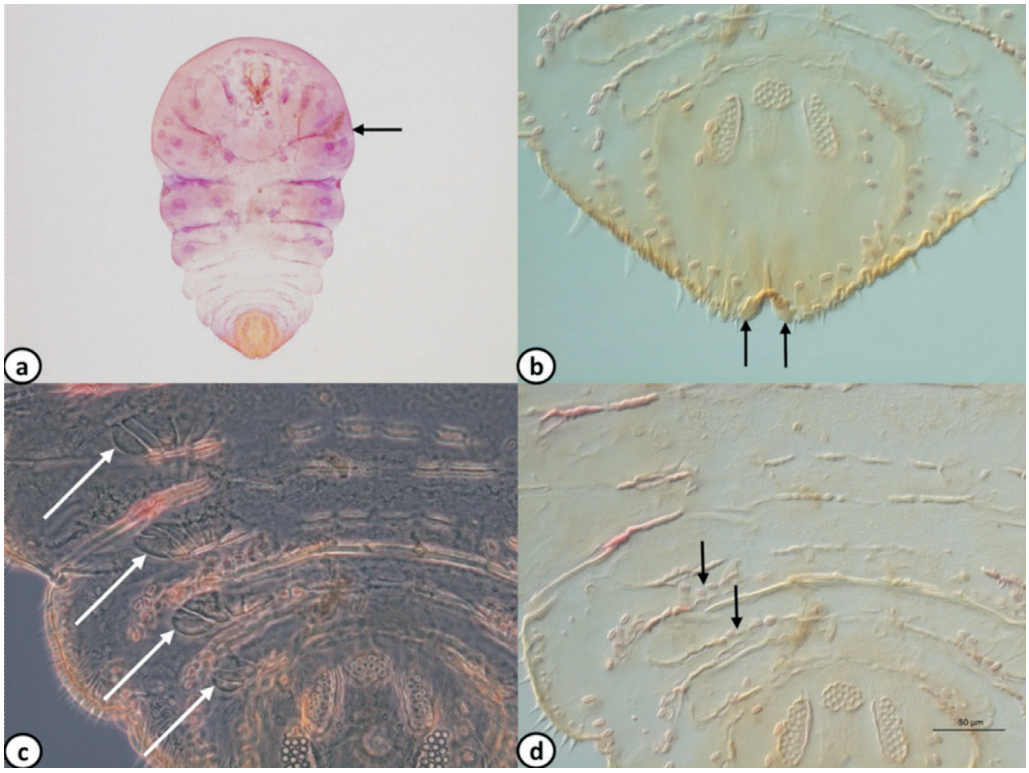

Fig. 2. Slide-mounted specimens of CAS. **a**, General body shape, showing rounded expanded prosoma (arrowed); **b**, median pygidial lobes (arrowed) forming notch; **c**, dermal ornamentation on ventral abdominal segments (arrowed); **d**, dorsal median macroducts present on the third and fourth abdominal segments (arrowed), but absent from first two abdominal segments.

prosoma of *A. yasumatsui* is distinctly more rounded than that of similar, common and widespread pest *Aulacaspis* species such as *A. crawii* (Cockerell), *A. maidiunensis* (Zehntner), *A. rosae* (Bouché), *A. rosarum* Borchsenius and *A. tubercularis* Newstead. In addition, CAS differs from these species by the combination of the diagnostic morphological features of the dorsal macroducts,

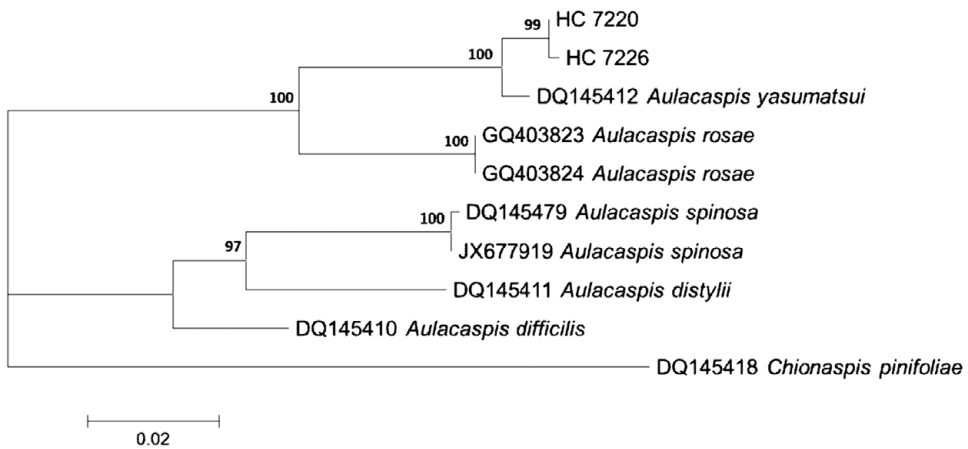

Fig. 3. Maximum likelihood (ML) phylogenetic tree based on *TEF1α* genomic DNA sequences showing identities of the scale insect samples *HC* 7220 and *HC* 7226. Numbers above branches indicate ML bootstrap values.

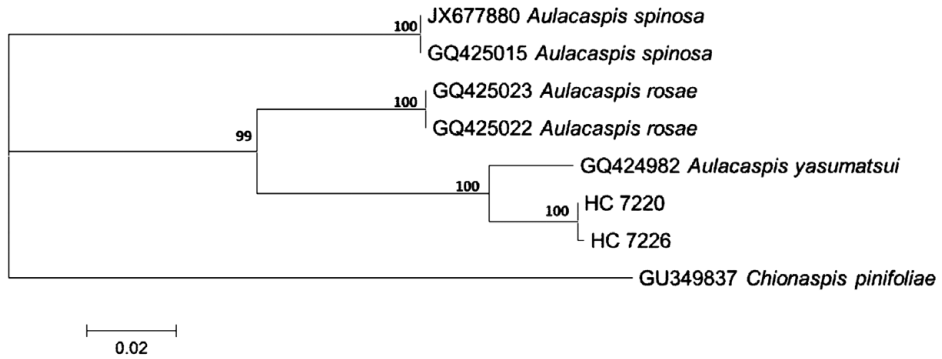

Fig. 4. Maximum likelihood (ML) phylogenetic tree based on *COI-COII* genomic DNA sequences showing identities of the scale insect samples *HC* 7220 and *HC 7226*. Numbers above branches indicate ML bootstrap values.

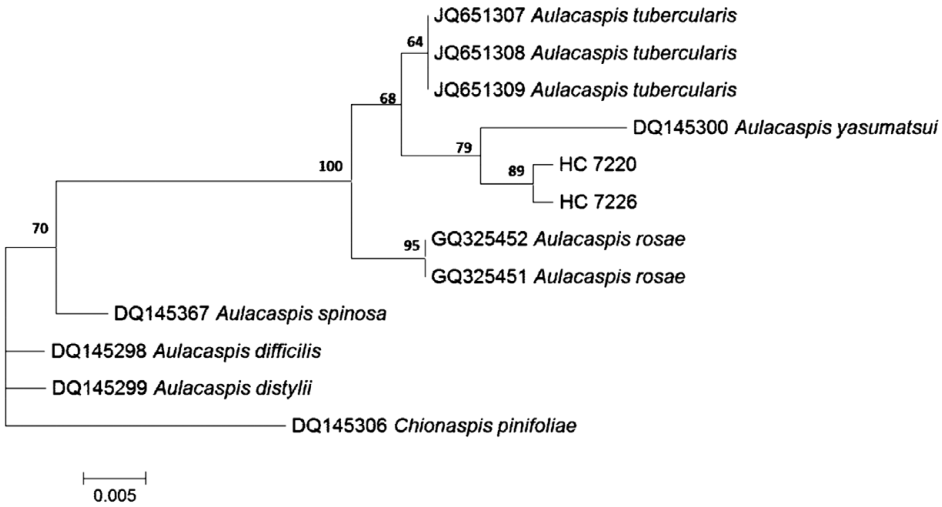

Fig. 5. Maximum likelihood (ML) phylogenetic tree based on *28S* genomic DNA sequences showing identities of the scale insect samples *HC* 7220 and *HC* 7226. Numbers above branches indicate ML bootstrap values.

pygydial lobes and dorsal microducts (Miller & Davidson 2005; Suh & Hodges 2007; Masten Milek *et al*. 2008; Suh & Ji 2009). Masten Milek *et al.* (2008) summarized the general morphological characteristics that separate CAS and *A. rosae*, such as scale appearance and body shape. Although *A. rosae* has been recorded from cycads (Miller & Gimpel 2009), its presence in South Africa has not been confirmed (Munting 1977).

Munting (1977) provided a taxonomic account of the South African species of *Aulacaspis*, in which he re-described and illustrated the three species that occur in the country (*A. crawii*, *A. maidiunensis* and *A. tubercularis*), and he provided a key for their identification. CAS tends to be intermediate to *A. maidiunensis* and *A. tubercularis* in this key, but it differs from *A. maidiunensis* by not having a fourth pair of clearly developed pygidial lobes, and from *A. tubercularis* by usually having more gland spines on the margin of abdominal segment four, and by the absence of projecting lateral sclerotizations on either side of the labium. Overall, CAS can be readily distinguished from the three other *Aulacaspis* species found in South Africa by the more rounded shape of the prosoma and the smoother margin of the abdomen. The prosoma of the other three species is more angular than that of CAS, and their anterior abdominal segments each form a more prominent lobe at the margin, compared with CAS (Masten Milek *et al*. 2008).

CAS is the only species of *Aulacaspis* that specifically feeds on gymnosperms, and is currently known to occur only on cycads (Takagi & De Faveri 2009). Thus, CAS should be relatively easy

to recognize, as the host plant association of any samples requiring diagnosis would normally be known, and this information, together with the distinctive body shape, makes this scale species readily identifiable. In the field, mature females of CAS can be recognized by their flat, white scale covers, which are circular to pear-shaped, 1.2 to 1.6 mm long and have small, light yellow or white exuviae of the immature stages embedded at the marginal areas of each cover. Often the cover is distorted due to conformation against adjacent leaf veins and crowding amongst other individuals. The adult female underneath the scale cover is yellow to orange, with distinctive swollen prosoma. Male scale covers are much smaller than those of the female, about 0.5 mm long. They are white, felted, elongate with parallel sides and bear three longitudinal ridges (tricarinate). The exuvium of the first immature stage is light yellow or white and is borne at one end of the scale cover. Adult males have wings and are tiny, delicate insects that resemble midges, but are short-lived and not often observed (Hodges *et al*. 2003; Miller & Davidson 2005; Malumphy & Marquart 2012).

Other species of armoured scales that regularly occur on cycads in South Africa include *Aspidiotus capensis* Newstead on *Encephalartos*, *Furchadaspis zamiae* (Morgan) on unidentified cycads and *Encephalartos*, and *Lindingaspis rossi* (Maskell) on *Cycas* and *Encephalartos* (SANC collection records). Slide-mounted specimens of these species can be readily distinguished from CAS by their clearly different body shapes, and pygydial features such as the arrangements of ducts and the appearance of the marginal lobes. In general, CAS infestations can usually be recognized as such by their severity, with colonies densely covering the affected parts of cycads, and the small narrow male covers being much more numerous than the rounded/oval female scales (Malumphy & Marquart 2012).

CAS can be distinguished in the field from other species of armoured scales that regularly occur on cycads in South Africa. *Aspidiotus capensis* has a white, circular scale cover and the rounded exuviae of earlier instars are borne on top in the centre of the mature scale. The body of the adult female is turbinate in shape. *Furchadaspis zamiae* also has a white scale cover, almost circular in shape, but the body of the adult female is turbinate. *Lindingaspis rossi* has a circular scale cover that is dark brown to black, and the body of the adult female is of a turbinate shape, clear to light pink in colour (Miller & Davidson 2005). A further scale species that may possibly be encountered on cycads in South Africa is *Pseudaulacaspis cockerelli* (Comstock), which also has a white scale cover, shaped like an oyster shell in the mature female. However, the body of the adult female is yellow and elongate-oval with a narrow prosoma, and looks clearly different to CAS (Hodges *et al*. 2003; Miller & Davidson 2005).

Molecular identification based on the 8S, TEF1α and COI-COII gene regions grouped the scale insect, observed on South African cycads, with *Aulacaspis yasumatsui* (CAS). The bootstrap value supporting this grouping on the 28S phylogenetic tree is, however, low and sequence differences exist between our specimens and those in the GenBank data base. For all three gene regions (28S, EF1α and COI-COII) only one sequence for *A. yasumatsui* occurs in GenBank and minor sequence differences were found between South African specimens of *A. yasumatsui* and those in GenBank (2 and 4 %, respectively). However, these are less than that observed between *A. yasumatsui* and other species of *Aulacaspis*. These differences may reflect population/geographic level variation between specimens. Additional specimens from other parts of the world will be needed to investigate the observed sequence differences in more detail and to investigate the possible presence of cryptic species in *A. yasumatsui*.

Aulucaspis yasumatsui has been recorded from all three cycad families, the Cycadaceae (Bailey *et al*. 2011; Howard *et al*. 1999; Heu 2002; Kozar *et al*. 2013; Malumphy & Marquart 2012; Takagi 1977), Stangeriaceae (Malumphy & Marquart 2012; Miller & Davidson 2005; Howard *et al*. 1999) and Zamiaceae (Halbert 1998; Hodgson & Martin 2001; Howard *et al*. 1999; Malumphy & Marquart 2012; Miller & Davidson 2005; Muniappan *et al*. 2012). All *Cycas* species (Cycadaceae) outside the native range of CAS are susceptible to infestation (IUCN/SSC-CSG 2005). These include *C. circinalis* (Malumphy & Marquart 2012), *C. media* (Howard *et al*. 1999), *C. micronesica* (Bailey *et al*. 2011), *C. panzhihuaensi* (Howard *et al*. 1999), *C. revoluta* (Howard *et al*. 1999), *C. rumphii* (Howard *et al*. 1999), *C. seemannii* (Howard *et al*. 1999), *C. szechuanensis* (Howard *et al*. 1999), *C. taitungensis* (Bailey *et al*. 2011), *C. thouarsii* (Howard *et al.* 1999) and *C. wadei* (Howard *et al*. 1999). However, in the absence of natural predators, cultivated *Cycas* plants, even in the native range of CAS, are also at

risk (IUCN/SSC-CSG 2005). CAS is also a pest to many other cycad genera, these include the genera *Bowenia* and *Stangeria* (Stangeriaceae) and genera belonging to the Zamiaceae family, *Dioon*, *Encephalartos*, *Macrozamia*, *Microcycas* and *Zamia* (http://www.sel.barc.usda.gov/scalecgi). In the current study CAS was observed infesting five *Encephalartos* species (*E. longifolius, E. natalensis, E. paucidentatus, E. transvenosus* and *E. villosus*) which are endemic to South Africa and which were previously not reported as hosts of CAS.

Management of CAS can be challenging, as the waxy protective cover that shelters these scale insects and their ability to burrow themselves into crevices on plants tend to make the application of traditional insecticides less effective (Song *et al*. 2012). The IUCN has, therefore, recommended several CAS control measures which include horticultural oils, pesticides, cultural methods, biological control agents, as well as integrating of all of these methods (Chamberlin 2005; Hodges *et al*. 2003; IUCN/SSC-CSG 2005; Wiese *et al*. 2005). The introduction of natural enemies as biological control agents has been recommended by the IUCN as the most cost- and labour-effective method for controlling CAS populations. The parasitic wasp *Coccobius fulvus* Compere & Annecke (Hymenoptera: Aphelinidae) and predatory beetle *Cybocephalus nipponicus* Endrödy-Younga (Coleoptera: Nitidulidae) are native to Thailand and natural predators of CAS. In 1998, these two insects were imported from Thailand and released in Florida as biological control agents for CAS (Song *et al*. 2012). It was observed that when they were used together, affected cycad hosts became almost free of scales (USDA 2002). The tiny black lady beetle, *Rhyzobius lophanthae* Blaisdel (Coleoptera: Coccinellidae), can also be used as an alternative to the parasitic wasp. The lady beetle is highly effective in controlling CAS and is recommended as the primary control strategy for cycad homeowners (Hara *et al*. 2005).

CAS can be transported long distances to new locations through the import and movement of infested cycad plants. The ability of this insect to 'hitch-hike' and remain undetected within stems and root systems of infected plants has allowed this scale insect to spread throughout many parts of the world as a result of an increase in cycad trade (Song *et al*. 2012). CAS crawlers (the dissemination life stage) can also easily spread within short distances to healthy plants through wind currents or pruning of infested plants (IUCN/SSC-CSG 2005). CAS has also been observed to infest parts of plants that are below ground level, such as the roots. This subterranean infestation can become a reservoir for re-infestation after infested plant parts have been removed (Howard *et al*. 1999).

In South Africa, non-native cycad species, *e.g*. *Cycas* species, may act as reservoirs of CAS and other pests/pathogens and, therefore, pose a threat to native cycad species. As stated by Marler & Moore (2010), the majority of the international cycad trade is dominated by *C. revoluta* plants. In the current study, infested *C. revoluta* and *C. thouarsii* plants were observed in two commercial nurseries. Whereas permits are required for the possession and movement of native South African *Encephalartos* species, no such control exists for non-native species. Regulations, therefore, need to be developed in order to limit the trade of susceptible non-native cycad species, and thus the spread of CAS and other non-native pests in the country.

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