The occurrence and characteristics of *Imbrasia belina* (Westwood, 1849) in the subtropical region of KwaZulu-Natal Province, South Africa

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Mopane worm is the edible larva of *Imbrasia* (*Gonimbrasia*) *belina* (Westwood, 1894), a species of emperor moth that is generally found in central and southern African tropical regions. Both over-harvesting of larvae and the destruction of the mopane woodlands are threatening its biodiversity. An insect with a description matching that of *I. belina* was observed in the northern coastal region of KwaZulu-Natal, a subtropical biota. The aim of this study was to gain insight into the potential of the northern coastal region of KwaZulu-Natal as a sanctuary for *I. belina*. The presence of *I. belina* in the subtropical biota of the coastal region of KwaZulu-Natal was confirmed through mitochondrion *CO1* gene sequences, this being so far its southernmost occurrence. Field surveys revealed the occurrence of four morphologically distinct variants within the uMkhanyakude District, inclusive of the protected iSimangaliso Wetland Park and Hluhluwe Game Reserve from the beginning of September to early November as do most of the populations in the mopane woodlands but differs from them by having one outbreak per season instead of two. *Imbrasia belina* is polyphagous and feeds off hosts including marula (*Sclerocarya birrea* [(A. Rich.) Hochst.] [Anacardiaceae]) and seven other tree species. There is therefore scope to use the northern KwaZulu-Natal coastal region as a sanctuary for biodiversity conservation of *I. belina*. There are initiatives to cultivate marula for its fruit in the region, which further increases the potential of the area as a sanctuary for *I. belina* by farming marula for both its fruit and *I. belina*. The protected nature reserves present in the region will ensure areas of controlled use by humans.

Key words: mopane worm, biodiversity, *Sclerocarya birrea*, over-harvesting, distribution.

INTRODUCTION

Imbrasia belina (mopane worm) (Westwood) (Lepidoptera: Saturniidae) belongs to the group of moths referred to as emperor moths (Pinhey 1972). Its distribution is mainly in the tropics and primarily occurs on the mopane tree (*Colophospermum mopane* (Benth.) J. Léonard), hence it is called mopane worm. However, it is not limited to *C. mopane* (Ditlhogo 1996). It has been observed feeding on numerous plant species which include *Carissa macrocarpa* (Eckl.) A.DC., *Diospyros* sp., *Ficus* sp., *Searsia lancea* (L.f.) F.A.Barkley, *Sclerocarya birrea* (A.Rich.) Hochst., *Terminalia sericea* Burch. ex DC., *Trema bracteolata* (Hochst.) Blume, *Brachystegia* spp. and *Julbernadia* spp. (Pinhey 1972; Allotey *et al*. 1996; Potgieter *et al*. 2012).

Traditionally, rural people have harvested and consumed *I. belina* as a nutritious protein resource (Makhado *et al*. 2012). However, in recent times, the status of *I. belina* has moved from being a food resource for poor rural folk to a commercial commodity worth millions of dollars (Thomas 2013; Baiyegunhi & Oppong 2016). It has now formed an extensive trading commodity within sub-Saharan Africa (Ditlhogo 1996) including South Africa, Zambia, Botswana, Namibia, Mozambique and Zimbabwe (Ditlhogo 1996; Kozanayi & Frost 2002). The commercialisation of *I. belina* has led to unsustainable harvesting practices, which threaten to severely deplete the populations of the insect in those areas where it is harvested (Sekonya *et al*.

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2020). Further, there are reports that in some areas there is reduction in the Mopane woodlands through cutting down of trees for firewood and clearing large tracts for growing crops (Mugari *et al*. 2019; Ndlovu *et al*. 2019). Recent extreme climatic events such as droughts and floods as well as poor management have also had a negative impact on the mopane worm (Sekonya *et al*. 2020). A severe decline in the populations of the insect will impact negatively on the livelihoods of the rural people who depend on it for food and trade. This emphasises the need to create sanctuaries of *I. belina* that are safe from exploitation, which can be used for re-stocking the overexploited area in addition to instituting sustainable harvesting regulations. Most desirably, sanctuaries need to be outside the commercial harvesting areas. In this respect, an insect with a description matching that of *I. belina* occurs in a subtropical region in the northern coastal area of KwaZulu-Natal (unpublished). The protected iSimangaliso Wetland Park falls within this area, and thus has potential to serve as a sanctuary for *I. belina*. Currently, there is no evidence that *I. belina* in this area is harvested for commercial purposes apart for household consumption by the local communities.

The study being described herein had three objectives. The first was to confirm the identity of the northern coastal KwaZulu-Natal organism as *I. belina*. The second was to investigate the extent of its occurrence and to identify its natural enemies and competitors in the region. The third objective was to gain insight into the potential of the northern coastal KwaZulu-Natal as a sanctuary for *I. belina*.

MATERIAL AND METHODS

Study site

The study was conducted in uMkhanyakude District (27.2719°S 32.5373°E) located in the north-eastern part of KwaZulu-Natal province (Fig. 1). The climate is sub-tropical with hot humid summers and mild winters. The winter temperatures range from 11 °C to 23 °C due to the warm Agulhas ocean current. The lowest temperatures are experienced between March and July. The mean daily maximum temperature in uMkhanya-

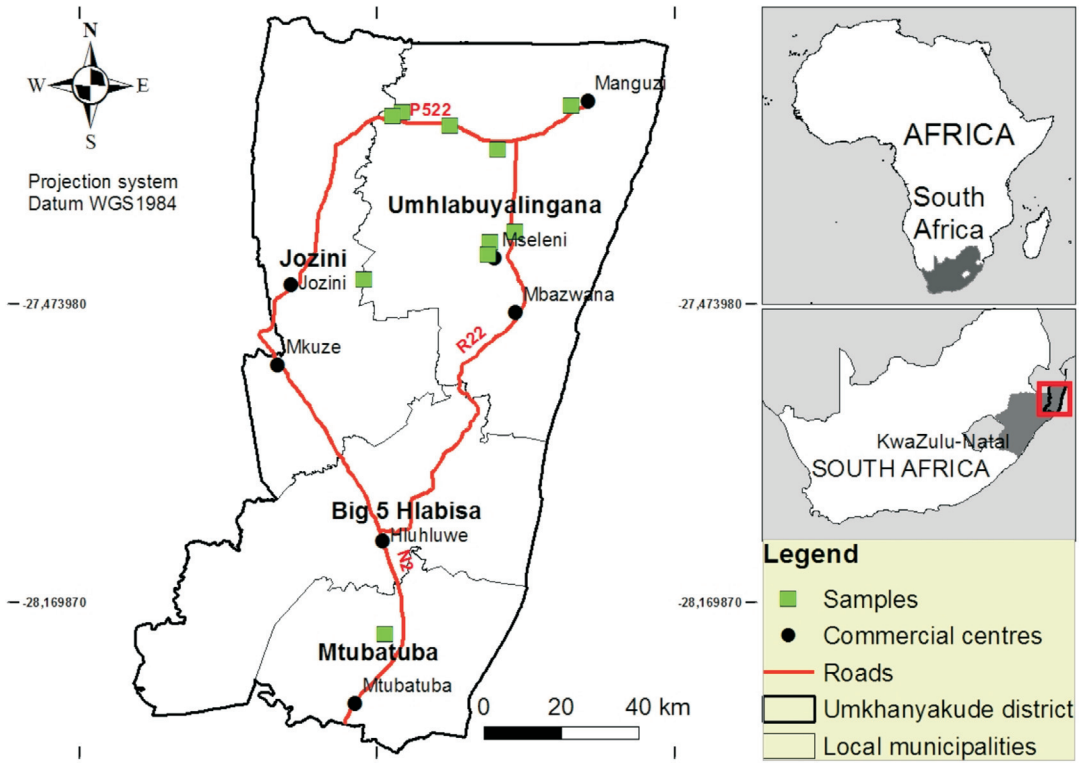

Fig. 1. Map of Umkanyakude district showing the survey sites and commercial centres in the district.

kude ranges from 21 °C in July to 26 °C in January. The mean daily minimum temperature ranges from 9 °C in July to 19 °C in January, with extremes falling to between 3 °C and 8 °C, respectively (Ndulini 2009). The area receives rainfall of between 750 and 1250 mm per annum throughout the year, but about 60 % of the rainfall occurs during the spring and summer months (September to March) (Ndulini 2009). The area is dominated by sandy soils, which are not suitable for most agricultural activities (Ndulini 2009). uMkhanyakude has six vegetation types, which include Savanna, wooded grassland, Azonal Forest, Forest, Indian Coastal Belt, and Wetlands (Ezemvelo KZN Wildlife 2014).

Sampling, amplification, sequencing, and processing of molecular data

Specimens (five per site) were collected from three widely separated communal areas in the uMkanyakude District, which included a site near Jozini town (27°24′52.2°S 32°13′50.8°E), a site near Skemelele township (27°02′08.2°S 32°14′33.5°E) and a site near Manguzi town 27°00′35.3°S 32°43′09.1°E). In addition, live specimens were obtained from local mopane worm collectors near Beit Bridge on the Masvingo-Beit Bridge road in Zimbabwe (21°53′20.9°S 30°19′28.4°E). All specimens were placed in 50-ml polycarbonate bottles containing absolute ethanol, and taken to the laboratory at the University of Zululand, Empangeni, KwaZulu-Natal, where they were stored at –80 °C in a cryogenic freezer until DNA sequencing.

DNA sequencing was performed from the mitochondrion CO1 (mtDNA CO1) gene region of one of the five specimens per site. The DNA was extracted from specimens using the Tissue Mini Prep kit from Zymo Research following the manufacturer's protocol. Polymerase chain reaction (PCR) was conducted with Lara C1 Sequence primers (Forward GRTCHCCWCCTCCTCYHGG RTC and Reverse GATTTTGATCAGGWATAC) in DreamTaq, 25 μl reactions with 10 pmol primer and around 30 ng of gDNA. The cycling protocols used were as follows: 95 °C for 5 min, 95 °C for 30 sec, 50 °C for 30 sec, 45 cycles, 72 °C for 1 min, 72 °C for 10 min, 4 °C hold. Successful amplicons were then purified using ExoSap following the manufacturer's protocol. The purified templates were sequenced using the ABI Big Dye kit v3.1. The sequenced products were cleaned with the Zymo sequencing clean-up kit before injection on ABI 3500 Xl genetic analysers with a 50 cm array and POP7. DNA sequences were manually edited (for base calling errors), pruned and aligned by ClustalW using the BioEdit Sequence Alignment Editor (Hall 1999) to create consensus sequences which were saved in the Fasta format in MEGA5 (Tamura *et al*. 2011). All consensus sequences were compared to reference sequences at the NCBI and the Barcode of Life Data System (BOLD), a repository for COI sequences.

Field survey for incidence of I. belina *and identification of host trees*

Two field surveys were conducted. The first was done from 30 October to 11 November 2016, and the second from 6 to 11 February 2017. The field surveys were accomplished through spot checks and transectional scouting to map out the spread of the area of the incidence of *I. belina* and to identify its host trees. *Sclerorcaya birrea* woodlands were the primary target during field scout visits. Spot checks were done along the N2 road from Mtubatuba to Jozini, starting at Nyalazi River bridge (28°14′34.0°S 32°17′07.6°E) in Mutubatuba, which coincided with the end of commercial *Eucalyptus* plantations. The spot checks were also conducted from Jozini to Manguzi along the P522 road and from Hluluwe to Manguzi along the R22 road after every 5 km when *S. birrea* plants were encountered on the road. In addition to these spot checks, walks were conducted along defined 1 km transects each at Makhatini, Lulwane, Kwandaba, Esicabazini, Nhlamvu (Mseleni), Jobe, Mzila primary school, Mangusi, Ndlondlweni. Since quantification of the host plants and infestation rate were not part of the study, only one transect per site was used to detect the occurrence of *I. belina*. The coordinates of the transects localities are shown in Table 1.

Imbrasia belina host trees were identified by the presence of live larvae or hatched eggs accompanied by larval droppings under the trees. Specimens of the host tree species utilised by *I. belina* were taken to and their identity confirmed by T.H.C. Mostert or R. Ntuli of the Department of Botany, University of Zululand. Edible caterpillars of other moths that were feeding on *S. birrea* and other host plants utilised by *I. belina* were also identified as potential competitors of *I. belina*.

Infestation level

Due to the drought that occurred at the end of

Table 1. GPS coordinates of the sites for transsectional scouting for the incidence of *Imbrasia belina* in uMkanyakude District.

Site	Coordinates
Makhatini (Mtinkwe)	27°24′55.4°S 32°14′09.1°E
Lulwane	27°02′02.8°S 32°18′14.2°E
Kwandaba	27°01′36.3°S 32°19′36.6°E
Esicabazini	27°03′25.7°S 32°26′16.7°E
Mseleni (Nhlamvu area)	27°18′18.8°S 32°35′20.8°E
Jobe	27°21′25.4°S 32°31′35.0°E
Mzila primary school	27°19′44.3°S 32°31′53.5°E
Manguzi (Thengani area)	27°00′33.8°S 32°43′14.5°E
Ndlondlweni	27°06′43.3°S 32°33′00.3°E

2016, *I. belina* did not emerge in most of the survey areas and was highly active only in a small area stretching for 3 km west of Manguzi town. Therefore, quantification of *I. belina* in the uMkhanyakude District was not possible. The level of infestation based on the number of plants that were infested were determined only in the small area near Manguzi town (27°00′33.8°S 32°43′14.5°E) in which the occurrence of the mopane worm was high. Nineteen marula plants were inspected for mopane worm infestation in a 190 × 25 m area. The number of egg clutches per tree were counted, and the egg clutches collected to determine the number of eggs per clutch and to measure the size of the eggs. It was assumed that each clutch of eggs was laid by one female, and that each female laid only one clutch of eggs. The number of eggs contained in each egg clutch was thus used to determine the average number of eggs laid by the females. The size of the eggs was measured in millimetres using callipers.

Presence of viral diseases and parasitoids

Imbrasia belina larvae were inspected for any diseases or parasitism and diagnosis was based on the literature on diseases and parasitoids associated with *I. belina* larvae (Ditlhogo 1996).

RESULTS

Mitochondrion *CO1* DNA analysis

Representative mitochondrion sequences of specimens taken from the study area and from Zimbabwe are presented in Table 2. The mitochondrion *CO1* gene sequences of the three larval specimens taken from the subtropics in the northern coastal region of KwaZulu-Natal had 99.24 to 100 % similarity with those of *I. belina*, and 99.24 to 99.74 % similarity with those of two other African Saturniidae moths, *I. belina* subsp. *osiris* and *I. ufipana* in the BOLD gene bank. The specimens collected from the tropics in the Beit Bridge area in Zimbabwe also had a similar match with sequences of *I. belina* (99.48 to 99.74 %), *I. belina* subsp. *osiris* (99.48 to 99.74 %) and *G. ufipana* (99.48 to 99.74 %) from the BOLD gene bank as displayed by the specimens from KwaZulu-Natal (Table 3). Both *I. belina* subsp. *osiris* and *G. ufipana* are African moths

The extent of the incidence of *I. belina* on the northern (Maputaland) coastal plain of KwaZulu-Natal

Larval feeding was observed only in the first survey (31 October to 11 November). In the second survey (20 February to 4 March 2017), there were no larval occurrences on the host plants identified in the first survey nor were there larval droppings under the host trees. In the first survey, although *S. birrea* and other host plants of *I. belina* were present between Empangeni to Hluhluwe, and between Mtubatuba and Mkuze, *I. belina* larvae were not on any of the trees inspected. Also, *I. belina* was absent on *S. birrea* trees between Hluhluwe and Mseleni on the R22 road. Along the N2 motorway, the incidence of live *I. belina* larvae feeding was observed just after Mkhuze (27°36′23.5°S 32°00′52.4°E). From Hluhluwe to Manguzi along the R22 road the incidence of *I. belina* larvae was first observed starting in the Jobe area (27°20′47.8°S 32°31′42.4°E). Feeding larvae were most prevalent in Makathini (27°24′55.4°S 32°14′09.1°E), KwaNdaba (27°01′36.3°S 32°19′36.6°E), Mseleni (27°18′18.8°S 32°35′20.8°E) and Manguzi (27°00′33.8°S 32°43′14.5°E). Of the areas surveyed/visited, the highest incidence of *I. belina* was in the area extending from Tengani (27°00′46.8°S 32°42′30.1°E) to Manguzi (26°59′42.4°S 32°44′33.1°E).

Host plant range

Imbrasia belina larvae were observed feeding on a number of tree species in the northern coastal region of KwaZulu-Natal (Table 4). An isolated incidence was observed in which *I. belina* larvae were observed feeding in Manguzi on mango (*Mangifera indica* L.), an exotic tree species. Other host plants included *S. birrea*, *Grewia* spp., *Vachellia*

Table 2. Representative mitochondrion *COI* sequences of *Imbrasia belina* specimens collected from Manguzi (subtropical region) in the northern coastal area of KwaZulu-Natal (**A**) and Beit Bridge area (tropical region) in Zimbabwe (**B**).

A) Mt COI sequence of a mopane worm specimen collected from Manguzi

GAAWWTAGCTTTCCCCCGAATAAATAATATAAGTTTTTGATTATTACCCCCTTCA
TTAATTCTATTAATTTTTAGTAGCATTGTTGAAAATGGRGCAGGAACTGGATGAA
CAGTATACCCCCCCATTATCTTCTAATATTGCCCATAGAGGTTCTTCCGTTGATTT
AACTATTTTTTCCTTACATTTAGCAGGAATTTCATCTATTCTAGGAGCTATTAATT
TTATTACCACAATTATCAATATACGAATAAATAATATATCTTTTGATCAAATACCAT
TATTCGTTTGAGCTGTAGGAATTACAGCTTTTTTACTTTTATTATCTCTTCCAGTT
TTAGCTGGAGCTATTACTATATTATTAACAGATCGAAATCTAAATACTTCATTTTT
TGAYMMRRGAGGAGGAGGRRMCMMAG

B. Mt COI sequence of a mopane worm specimen collected from Beit Bridge area in Zimbabwe

GGGTCAGATATAGCTTTCCCCCGAATAAATAATATAAGTTTTTGATTATTACCCC
CTTCATTAATTCTATTAATTTTTAGTAGCATTGTTGAAAATGGAGCAGGAACTGG
ATGAACAGTATACCCCCCATTATCTTCTAATATTGCCCATAGAGGTTCTTCCGTT
GATTTAACTATTTTTTCCTTACATTTAGCAGGAATTTCATCTATTCTAGGAGCTAT
TAATTTTATTACCACAATTATCAATATACGAATAAATAATATATCTTTTGATCAAAT
ACCATTATTCGTTTGAGCTGTAGGAATTACAGCTTTTTTACTTTTATTATCTCTTC
CAGTTTTAGCTGGAGCTATTACTATATTATTAACAGATCGAAATCTAAATACTTCA
TTTTTGAYMMRRGAGGAGGRGGGRRMCMMARGAGAAGG

karroo (Hayne) Banfi & Glasso, *Trema orientalis* Linn. Blume, *Schotia brachypetala* Sond., *Sclerocroton integerrimus* Hochst., *Saba comorensis* (Bojer) Pichon and *Burkea africana* Hook. There were, however, geographical differences in the host plant range (Table 3). Outside Manguzi town (between Manguzi and Tengani), the larvae were observed feeding on *S. birrea*, *Grewia* spp., *S. integerrimus*, *T. orientalis*, *V. karroo* and *M. indica*. In KwaNdaba, 51 km away from Manguzi, *I. belina* was observed feeding on *B. africana*, *S. brachypetala*, *S. comorensis*, and *S. birrea*, but not on *V. karroo*, *M. indica*, and *Grewia* spp. that were also present. At Makatini, *I. belina* was observed feeding only on *B. africana*, *S. comorensis* and *S. birrea* even though the other host plants mentioned herein were present. In Mseleni, it was observed feeding only on *S. birrea*, *S. comorensis* and *V. karoo*. The common

Table 3. Percentage similarity of mitochondrion *COI* sequences of specimens collected from Beit Bridge in Zimbabwe (ZIM) and northern coastal region of KwaZulu-Natal province in South Africa (KZN) with sequences in the BOLD gene bank.

Specimen	Similarity with *I. belina* (%)	Similarity with *I. belina* subsp. *osiris* (%)	Similarity with *G. ufipana* (%)
Specimens obtained from Beit Bridge area in Zimbabwe (ZIM)			
ZIM 1	99.48	99.48	99.48
ZIM 2	99.74	99.74	99.74
ZIM 3	100	99.74	99.74
Specimens obtained from KwaZulu-Natal (KZN) northern coastal region			
KZN 1	100	99.74	99.74
KZN 2	99.24	99.24	99.24
KZN 3	99.47 to 100	99.47	99.47

Table 4. Plant species on which *Imbrasia belina* larvae were observed feeding in uMkanyakude District in the subtropical region in the northern coastal plain of KwaZulu-Natal.

Site	Coordinates	Host plant species
Makhatini	27°24′55.4°S 32°14′09.1°E	*Sclerocarya birrea*, *Saba comorensis*, *Burkea africana*
Lulwane	27°02′02.8°S 32°18′14.2°E	*Sclerocarya birrea*, *Saba comorensis*, *Schotia brachypetala*
Kwandaba	27°01′36.3°S 32°19′36.6°E	*Sclerocarya birrea*, *Schotia brachypetala*, *Burkea africana*
Esicabazini	27°03′25.7°S 32°26′16.7°E	*Sclerocarya birrea*
Mselen (Nhlamvu area)	27°18′18.8°S 32°35′20.8°E	*Sclerocarya birrea*
Jobe	27°21′25.4°S 32°31′35.0°E	*Sclerocarya birrea*, *Acacia karoo*
Mzila primary school	27°19′44.3°S 32°31′53.5°E	*Sclerocarya birrea*, *Acacia karoo*
Manguzi	27°00′33.8°S 32°43′14.5°E	*Mangifera indica*, *Grewia* spp., *Trema orientalis*, *Acacia karoo*, *Sclerocroton integerrimus* (= *Sapium integerrimum*)
Ndlondlweni	27°06′43.3°S 32°33′00.3°E	*Vachellia karoo*

host tree species among all locations was *S. birrea*.

A patch of land with a high density of *S. birrea* plants located about 3 km west of the town of Manguzi (27°00′33.8°S 32°43′14.5°E) had the most infested plants. Of the 19 *S. birrea* trees that were inspected in this area (190 m × 25 m), only five of the trees were not infested. Those that were not infested were small and less than 3 m tall. Five of the plants were completely defoliated, and the larvae gone (their droppings were observed on the ground). Nine of the plants still had some larvae feeding on them.

Other edible moth species found feeding on host plants of *I. belina*

Two other edible moths (Lepidoptera: Saturniidae) found in uMkanyakude District were the cabbage tree emperor moth *Bunaea alcinoe* (Stoll) and *Imbrasia forda* (Westwood). Whilst the cabbage tree emperor moth was found feeding on Natal mahogany (*Trichilia emetica*), *I. forda* was found on *S. birrea* and *B. africana*.

Colour polymorphism in *I. belina* larvae

There were colour variations among *I. belina* (Fig. 2). The variations occurred between broods, but not within the brood. There were four colour variants of *I. belina* larvae. 1) Black-cephalous-variant with thick black spines in rows running along the top and sides of the body as well as grey and yellow bands with black spots across the body. In addition, the body was marked with conspicuous red, yellow and black concentric rings (Fig. 2A). 2) Black-cephalous-variant with large spines, the red and black concentric rings were absent, yellow rings were present, the broad yellow bands with black spots were absent (Fig. 2B). 3) Red-cephalous-variant (Fig. 2C) had a red head and thick spines. 4) A fourth variant (not shown) was similar to the larva shown in Fig. 2A, except that it had red spines and a red head. In all variants, there were conspicuous triangular marks along the dorsal surface of the body.

The occurrence of both *I. belina* and *I. forda* coincided with the onset of summer rainfall (October to November), emerging only after the host plants had produced new leaves. There was no secondary emergence of *I. belina* in February/March as expected from the known life-cycle of the insect in the tropical region.

Characteristics of *I. belina* and *I. forda* eggs

Observed on the infested trees were hatched egg clusters. Both *I. belina* and *I. forda* laid eggs either on a leaf surface or twig. The eggs were laid in clutches. There were no more than three egg clutches laid per tree. Even after hatching, the eggs remained in the clutch. *Imbrasia forda* laid more eggs per clutch than did *I. belina*. Egg count per clutch for *I. belina* ranged from 101 to 216, and for *I. forda* it was from 130 to 287. *Gonombrasia belina* eggs were whitish, oval shaped, measuring an average of 3.06 mm × 1.94 mm. Those for *I. forda* were also oval, but were dirty white in colour. They were also smaller than those of *I. belina* eggs, with mean measurement of 2.04 mm × 1.46 mm.

Parasites and diseases

One parasite species was observed on both *I. belina* and *I. forda* larva. The parasite, a hymenopteran,

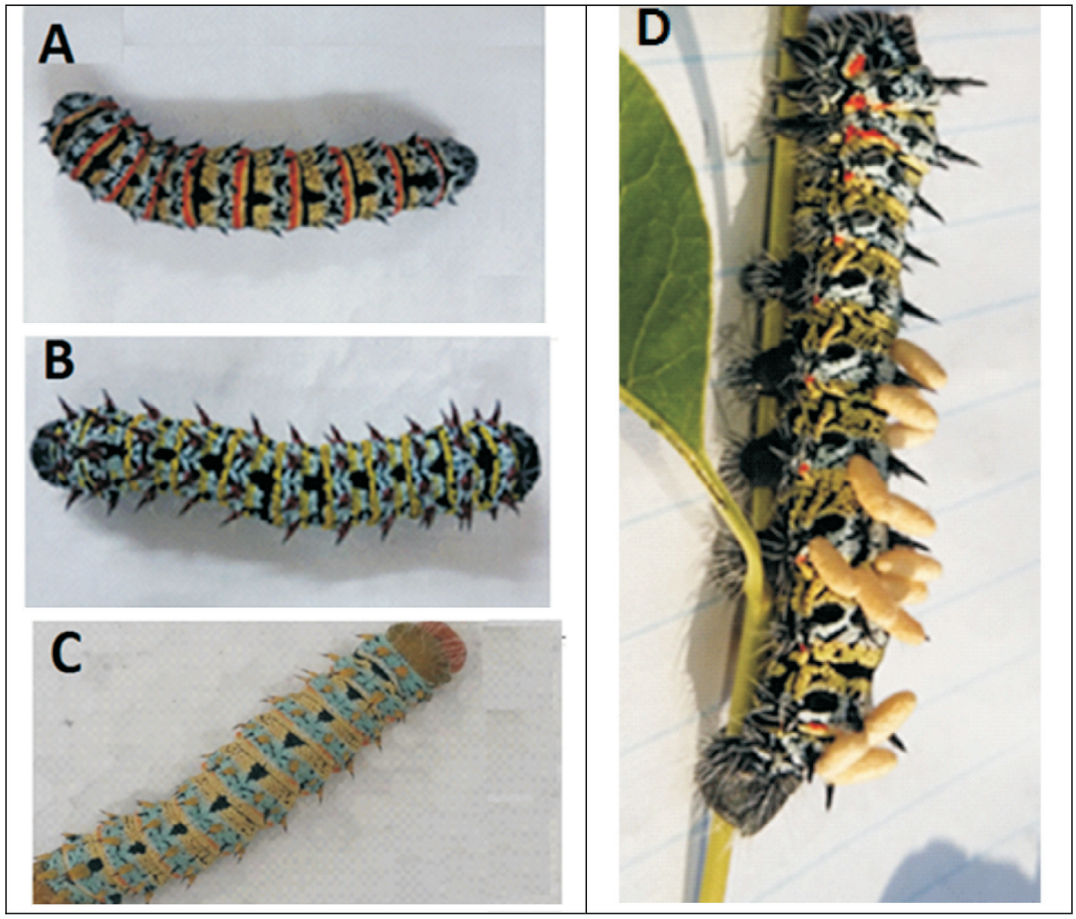

Fig. 2. Colour pattern variants of *Imbrasia belina* larvae (**A**, **B** and **C**) and cocoons of hymenopteran parasitoid on *I. belina* larva (**D**). Note the conspicuous red, yellow and black concentric bands/rings in (**A**), the absence of the red band/ring in (**B**) and the absence of both the black and red bands/rings in (**C**).

left dirty white cocoons on the bodies of the larvae (Fig 2D). The parasitism was, however, not widespread as it was observed only in one larval brood of *I. belina* and two larval broods of *I. forda*.

In a few cases, some of the larvae were found dead on the host tree hanging on a branch by legs. They dried out on the tree as a result of infection by nuclear polyhedrosis virus *Iridovirus*. Although the larvae were not taken for diagnosis, the symptoms of the virus are known.

DISCUSSION

Confirmation of the identity of *I. belina*

Generally, *I. belina* occurs within the tropical ecoregion dominated by mopane trees (*Colophospermum mopane*), its primary host plant. It has not been reported to occur outside the tropics. The mitochondrion *CO1* gene sequences of both mopane worm specimens taken from the tropics in the Beit Bride area in Zimbabwe and the specimens from the subtropical region in uMkhanyakude had similarities ranging from 98.98 to 100 % with sequences of *I. belina* in the BOLD gene banks. This was confirmation that the insect occurring in the subtropical region along the northern coastal area of KwaZulu-Natal and identical in description to *I. belina* is indeed *I. belina*. Hence, the presence of *I. belina* on the northern coastal belt of KwaZulu-Natal is its southernmost occurrence known so far, and is evidence of it occurring outside the tropics and far removed from the mopane ecosystem.

Another observation of interest is that the

sequences of the specimens from both uMkhanyakude and Beit Bridge had similar matches with *I. osiris* Druce, 1896 occurring in DR Congo, Kenya, Nigeria, Tanzania (GBIF Secretariat 2019) and *I. ufipana* Strand, 1911 occurring in Tanzania (Myers *et al*. 2020) as they did with *I. belina* sequences in the BOLD gene bank (Table 1). GBIF Secretariat (2019) has listed *G. osiris* as a subspecies of *I. belina*. Similarly, Kitching *et al*. (2018) have listed *G. ufipana* as a synonym of *I. belina*. The results of the mitochondrion *CO1* DNA analyses in the present study indeed confirmed that *Imbrasia osiris* Druce, 1896, *G. ufipana* Strand, 1911 and *I. belina* Westwood, 1894 are conspecifics.

There was polymorphism in the colour patterns among the mopane larvae, but this was evident only between broods, whereas, larvae from the same brood had the same colour patterns. The high colour polymorphism among the broods is most likely a defence strategy to reduce potential predation from visually oriented predators by preventing predators from developing a search image for any particular morph (Farallo & Forstner 2012).

Mopane worms are known to be bivoltine. That is, they produce two generations per rainy season. However, they can be univoltine, depending on rainfall and other climatic factors (Sekonya *et al*. 2020). In drought years there is no second outbreak and in arid regions such as parts of Namibia they are generally univoltine (Thomas 2013). After the first generation of mopane worms which occurred in the months of October and November, there was no second generation observed in the current study. The occurrence of a single outbreak in this study could have been anomalous, due to the drought in KZN during the study period.

Host trees

Although *C. mopane* is known as the primary host for *I. belina*, it is known to feed on other tree species (Van Voorthuizen 1976; Mughogho & Munthali 1995; Pinhey 1972; Pinhey 1975; Ditlhogo 1996). On the northern coastal region of KwaZulu-Natal, it feeds on a number of trees as an adaptation to the absence of *C. mopane*. It is not clear how *I. belina* selects it hosts among a vast number of trees, but it does show preferentialism in host selection. Whilst *I. belina* occurred on a significant number of different tree species, *S. birrea*, one of the most dominant tree species in the area, was infested in all locations in which *I. belina* occurred. The other host plants for *I. belina* observed in this study were *M. indica*, *Grewia* spp., *V. karoo*, *T. orientalis*, *S. brachypetala*, *S. intergerimus*, *S. comorensis*, and *B. africana*. Of these, *I. belina* has been previously reported to browse on *S. birrea*, *S. brachypetala* and *V. karoo* elsewhere (Gardiner 2002; Van Voorthuizen 1976; Mughogho & Munthali 1995; Pinhey 1972; Pinhey 1975; Ditlhogo 1996). These observations confirm the complex polyphagous nature of *I. belina* on one hand. On the other hand, the common occurrence of *I. belina* in all areas where walk inspections were carried out reveal that a special relationship exists between *I. belina* and *S. birrea* in the study area. In Botswana, *I. belina* population occurring in Serule where the main host plant *C. mopane* is not available, *Terminalia sericea* Burch. was the main preferred host, but when presented with *T. sericea* and *C. mopane*, the latter was the preferred host (Ditlhogo 1996). This shows that there is a mechanism that facilitates the selection of a host plant by *I. belina*. In the present study, there were notable geographic differences in the host plant range over as short a distance as 51 km, indicating that the selection mechanism could also be site-specific or site-dependent. This study also reveals that there are no host plant-related barriers to territorial expansion of *I. belina*. This is further affirmed by the fact that *I. belina* was observed feeding on an exotic plant species, *M. indica*, which is, however, related to *S. birrea*. This finding is of important significance with respect to both the conservation and the possible commercialisation of mopane worms. In the mopane woodlands where mopane worms are currently being commercially harvested, their populations are declining due to overharvesting coupled with climate change-related droughts and anthropogenic destruction of the woodland (Sekonya *et al*. 2020). Thus, the expanded distribution of *I. belina* to the subtropics and its occurrence on plant species other than mopane, raises the possibility of domesticating and commercialising this important resource for rural livelihoods outside the mopane woodlands. This will also relieve the pressure off the mopane woodlands, and at the same time provide a livelihood resource to communities outside the mopane woodlands. The ability of *I. belina* to adapt to subtropical climatic conditions and its polyphagous nature also signify that there is potential to farm *I. belina* on a number of plants and to extend it to the warm subtropical regions outside Africa.

Under natural conditions, the population of the host plants in KwaZulu-Natal is too sparse to support intense outbreaks required for viable commercialisation of the mopane worms. However, the occurrence of *I. belina* on *S. birrea* could be taken advantage of in uMkhanyakude District, where initiatives of cultivating *S. birrea* in orchards for its fruit have started. The orchards can be used to farm *S. birrea* for both its fruit and *I. belina* in addition to increasing the potential of the area as a sanctuary for *I. belina*.

At Manguzi, *I. belina* seemingly chose the host plants to colonise based on the forage content. It avoided the small *S. birrea* trees less than 3 m in height. This might be taken as evidence that the females exercise selection of host plant at the individual tree scale (Hrabar *et al*. 2009). The avoidance of small trees (under 3 m), in the present study could be a strategy to ensure adequate forage for the larvae, as it would run out before the pupation stage on small trees. Hrabar *et al*. (2009) conducted an in-depth study on tree selection by *I. belina* and also concluded that in the selection of plants for larval feeding, *I. belina* moths chose large trees, and that resource quantity, rather than quality was the primary determinant of host plant preference by the mopane moths.

Competition from other phytophagous insects

Imbrasia belina larvae were not the only edible Lepidoptera occurring on the northern coastal region of KwaZulu-Natal. Also feeding on *S. birrea* and on *Burkea africana* was a close relative, *I. forda*. Though *I. forda* and *I. belina* larvae fed on *S. birrea* and on *B. africana*, surprisingly, there were no instances where the two were found co-existing on the same individual tree. They targeted different individual plants and avoided host plants that were occupied by the other. This suggests that both *I. belina* and *I. forda* have an ability to sense the presence of broods (eggs and larvae) of the other and avoid occupying the same tree to reduce the competition for resources between their offspring. This phenomenon is commonly mediated through host-marking pheromones whereby chemical compounds produced by females are deposited on or into hosts to signal host occupancy to conspecifics or closely related species (Chen & Cheng 2005). The exclusion host occupancy observed between *I. forda* and *I. belina* in the present study suggests an existence of a powerful pheromone for host-marking discrimination between the two species (Chen & Cheng 2005).

Fecundity of *I. belina* and *I. forda*

It was assumed that an egg clutch was laid by a single female and that each female has the capacity to lay a single clutch of eggs (Ditlhogo 1996). Similar to observations made by Hrabar *et al*. (2009), there were no more than three egg clutches observed per tree. Few egg clutches per tree could be a result of conspecific exclusion mediated through hormone host marking discrimination to avoid over-infestation of hosts by repeated egg-laying into an already occupied/used host on one hand. On the other hand, it could also signify that fertility among *I. belina* females is very low. Whatever is the dominating factor between these two, this low number of egg clutches per tree turns out to be advantageous for the preservation of the species as it prevents larval overpopulation on the trees. An overpopulation of the larvae would be disastrous as it would result in shortage of food before the larvae reach pupation stage. The complete host tree defoliation near Manguzi is an example of such a catastrophe. In uMkhanyakude District, the host plants are sparsely populated and not as closely clustered as they are in the mopane woodlands. Thus, when they completely defoliate the host plant on which they were hatched before reaching pupation stage, the *I. belina* larvae do not, in most of the cases, find another suitable host plant nearby. The larvae therefore starve to death.

The dimensions of *Imbrasia belina* eggs at 3.06 mm × 1.94 mm in the present study were similar to 3.14 ± 0.26 mm by 1.84 ± 0.15 mm reported by Dube & Dube (2010), and were bigger than those for *I. forda*, which were 2.04 mm × 1.46 mm. It is not clear what factors determine the size of the eggs, but the size of the eggs may have a bearing on the number of the eggs. For example, *I. belina* which had bigger eggs laid significantly fewer eggs per clutch than did *I. forda*. Egg count per batch/clutch for *I. belina* ranged from 101 to 216, and for *I. forda* it was from 130 to 287 per clutch. The range of egg number obtained for *I. belina* in the present study is within the range of 50–200 eggs reported by other researchers (Gondo *et al*. 2010; Potgieter *et al*. 2012; Dube & Dube 2010). Ditlhogo (1996) reported that fecundity is related to the final weight of the insect before pupating. Unfortunately, in this study the weight of the

insects that laid eggs were not known, and is a factor to be considered in future research.

Parasitism and diseases of *I. belina*

Both *I. belina* and *I. forda* were prasitised by a hymenopteran parasitoid. The parasitoid, whose cocoons were observed on the bodies of *I. belina* and *I. forda*, have been reported in previous studies (Ditlhogo 1996). The parasitoid does not kill the larvae immediately. However, it parasitises its host in a way that interferes with normal growth of the host. In addition to parasitism *I. belina* and *I. forda* larvae were also vulnerable to a viral disease known as nuclear polyhedrosis virus (NPV) (Ditlhogo 1996). When infected by NPV, a larva may be found hanging on a tree by its legs (Smirnoff 1965; Evans & Allaway 1983; Steinhaus 1963), and this was the case observed in this study.

CONCLUSION

The mitochondrion *CO1* gene analyses confirmed that *I. belina* is indeed present in the northern coastal subtropical region of KwaZulu-Natal, this being its southernmost occurrence so far. *Sclerocarya birrea* was identified as its universal plant host in this region, but was also observed feeding on seven other tree species. Thus, *I. belina* is a polyphagous insect adapted to a wide range of climatic conditions, and does not seem to have host plant-related barriers that can impede its territorial colonisation in southern Africa. The larvae of the *I. belina* population in the subtropical region of KwaZulu-Natal are susceptible to the same parasitoids and fungal diseases that infect populations in the tropical regions. This population does not seem to have a secondary incidence in the February–March period which has been observed for some of the populations in the tropical regions.

Because of the absence of commercial harvesting of *I. belina*, there is scope to use the northern KwaZulu-Natal coastal region as a sanctuary for biodiversity conservation of *I. belina*. The protected nature reserves present in the region will ensure areas of non-exploitation by humans. There are also initiatives on cultivation of marula in the region for its fruit, which further increases the potential of the area as a sanctuary for *I. belina* by farming marula for both its fruit and for *I. belina*. In this regard, it might be necessary to determine the genetic diversity of the *I. belina* population in the northern coastal region of KwaZulu-Natal to assess its resilience.

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