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A Redescription of Spironoura duyagi from the Stomach of Malayan Box Turtles (Cuora amboinensis)

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A REDESCRIPTION OF SPIRONOURA DUYAGI FROM THE STOMACH OF MALAYAN BOX TURTLES (CUORA AMBOINENSIS)

(TITLE)

BY

REX A. KUYE

B.S., Eastern Illinois University, 1978

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A REDESCRIPTION OF SPIRONOURA DUYAGI FROM THE STOMACH OF MALAYAN BOX TURTLES (CUORA AMBOINENSIS)

ABSTRACT: Two hundred and twenty-six worms taken from the stomachs of three Malayan box turtles (Cuora amboinensis) were studied. The worms were identified as Spironoura spp. and their numerical measurements and morphological details compared to other described species in the genus. The nematodes, generally, conformed to published descriptions of Spironoura duyagi, but varied so widely with regards to presence or absence of key diagnostic features that a complete taxonomic analysis was undertaken.

Classification of the nematodes has been a cause for concern and controversy among zoologists since the late eighteenth century to the present (Yorke and Maplestone [1926] 1962; Hyman, 1951; Cheng, 1973; Schmidt and Roberts, 1977; Levine, 1968).

Despite modern scientific refinements, taxonomical inconsistency has persistently appeared in the work of some recent authors. Yorke and Maplestone [1926] 1962 put the nematode family Kathlaniidae, Atractidae, Cruziidae, Oxyuridae, Heterakidae and Subuluridae in superfamily Oxyuroidea. Chitwood and Chitwood [1950] 1974 placed Kathlaniidae together with Cosmocercidae, Heterakidae and Ascaridae in superfamily Ascaridoidea, Yamaguti (1961) classified Kath-

laniidae, Atractidae, Cruziidae and Oxyuridae in order Oxyuridea; but Chabaud (Grassé, 1965) relegated Kathlaniidae Cosmocercidae and Atractidae to superfamily Cosmocercoidea.

Yamaguti (1961) classified Spironoura, first described by Leidy in 1856 from the stomach of Emys serrata and the caecum of Cistudo carolina, as the only genus of subfamily Spironourinae; while Yorke and Maplestone [1926] 1962, had earlier established it as one of the four genera of subfamily Kathlaniinae. But the genus Spironoura has variously been referred to as Ascaris by Rudolphi in 1819 (Yorke and Maplestone [1926] 1962); Leptodera (Baird, 1858; Walton, 1932); Spirura by Diesing in 1861 (Yorke and Maplestone [1926] 1962); Nematoxys by Linstow in 1907 (Khalil, 1962); Oxysoma (Stewart, 1914); Falcaustra by Lane in 1915 (Yorke and Maplestone [1926] 1962); Florencioia by Travassos in 1919 (Yorke and Maplestone [1926] 1962; Spectatus by Travassos in 1923 (Yorke and Maplestone [1926] 1962); Falcustra by Walton in 1929 (Yamaguti, 1961); Nematoxynema by Skrjabin and Schikhobalova in 1951 (Khalil, 1962) and Velariocephalus (Singh, 1958; Khalil, 1962).

Most of the forty-five species discovered earlier are parasites of reptiles or occasionally of amphibians (Yorke and Maplestone [1926] 1962; Yamaguti, 1961); but, new species are increasingly being discovered from African and Asian fish (Campana-Rouget, 1961; Khalil, 1962, 1970; Soota and Chaturvedi, 1971; Vassiliades and Troncy, 1973; Petter, 1979). The spironourans have been considered primarily

parasites of reptiles, amphibians and more recently fish; but Jehan (1970a) found Spironoura cameroni to occur in the common quail (Coturnix coturnix).

Because the worms in this study conformed generally to published descriptions of Spironoura duyagi but differed widely with regards to key diagnostic features, it became necessary that a further taxonomical investigation be undertaken.

MATERIALS AND METHODS

Nematode worms previously extracted from the stomach of Malayan box turtles (Cuora amboinensis) and preserved in 70% ethyl alcohol were utilized in this study.

In the laboratory, the specimens to be examined were flooded with tap water to wash out traces of the preservative and transferred into petri dishes containing lactophenol solution. After clearing in lactophenol for 24 hours, the specimens were transparent enough for microscopic examination.

Specimens were mounted singly on serially numbered microslides with 1.00 ml lactophenol used as mounting medium and covered with coverslips.

Using a compound microscope, the mounted worms were sexed and the morphology of each studied.

Measurements of systemic organs at 100 x magnification was undertaken on each of the specimens using a compound microscope and ocular micrometer. To limit bias, the length, width, and where possible, height of a whole organ, or part of an organ, was measured separately and the data recorded in microns. The arithmetic mean for all collected data was computed.

Enface mounts were made using a glycerine jelly technique. Six whole and four enface mounts were selected for photographs. Line drawings of key diagnostic features were prepared.

The worms were identified to genus according to the descriptions in Leidy, (1856); Yorke and Maplestone, [1926] 1962; and Yamaguti, (1961). They were identified to species after studying the works of Baylis (1920), Boulenger (1923), Karve (1927), Tubangui and Villaamil (1933), Chatterji (1936), Mackin (1936) and Sathananthan (1972).

RESULTS

General Characteristics

Two hundred and twenty-six nematodes from Cuora amboinensis were examined. The body of the nematodes is covered with cuticle, having transverse striae but without lateral alae. The head is globular and is joined to the body without a distinct neck. The diameter of the head at its broadest part measures 0.17-0.20 mm (average, 0.18 mm) in the male and 0.19-0.22 mm (average, 0.18 mm) in the female (Figure 1).

The mouth cavity is surrounded by three lips, one dorsal and two subventral, and are supported by a cuticularized ring. The lips are flattened anterior-posteriorly and when viewed laterally are transparent and appear to project about 0.026-0.035 mm (average, 0.032 mm) in front of the head. Each lip bears two papillae. The nerve pulp of the papillae is bifurcated and when observed anteriorly, each of the outer branches of the pulp terminate in a papillus on the anterior surface of the lips. The ends of the inner branches are not visible. A pair of semi-circular structures are located on each side of the head, close to an outer branch of a subventral lip papillus. They are light, slightly convex and appear to be amphids (Figure 2).

The buccal cavity is shallow, slightly larger in the female than in the male. The pharnyx is short but distinct from the rest of the esophagus which is longer and more cylindrical. The esophagus

ends in a distinct posterior bulb having a tri-valvular apparatus. This principal bulb is separated by a constriction from a smaller anterior bulb, the two constituting a characteric "hourglass" configuration. At its posterior fifth, the cylindrical esophagus narrows in diameter until it joins the anterior bulb. The entire esophagus, from the extremity of the head to the tip of the trivalve of the posterior bulb is muscular and pigmented (Figure 1).

The intestine is expanded at its beginning where it engulfs the trivalve of the posterior esophageal bulb but is compressed by the gonads as it progresses posteriorly. It too shows some pigmentation. The intestine ends in a pyriform proctodeum (Figure 3).

The pigmented arcade cells extend for some distance posterior to the nerve ring and mask it. No trace of an excretory pore or cervical papillus is evident (Figure 1).

The posterior extremity of the worm is ventrally curved, more so in the male than in the female. There are no caudal alae. The tail ends in a point in both sexes but it is longer and more slender in the female.

Male (n = 137)

Males are smaller and more slender than females. The body is 6.0-9.7 mm long (average, 7.4 mm) by 0.44-0.51 mm wide (average, 0.49 mm) at the level of its greatest width. From the globular head, the buccal cavity is 0.03-0.04 mm (average, 0.03 mm) long and

0.09-0.12 mm (average, 0.10 mm) wide; followed by a slightly larger pharynx measuring 0.04-0.05 mm (average, 0.04 mm) in length and 0.08-0.13 mm (average, 0.09 mm) in width. The rest of the esophagus is 1.15-1.20 mm (average, 1.17 mm) long and 0.14-0.16 mm (average, 0.15 mm) wide but decreases to a width of 0.12-0.13 mm (average, 0.12 mm) for the posterior 0.19-0.24 mm (average, 0.22 mm) of its length before joining the "hourglass" section of the esophagus. The prebulb is 0.11-0.15 mm (average, 0.12 mm) long and 0.13-0.16 mm (average, 0.14 mm) wide while the post bulb measures 0.27-0.30 mm (average, 0.28 mm) long and 0.25-0.28 mm (average, 0.26 mm) wide.

The nerve ring, when distinguished, lies 0.17-0.40 mm (average, 0.31 mm) from the cephalic end.

The masculature of the posterior extremity is well developed and bears two fan-shaped, preanal, pseudosuckers on the ventral surface. The anterior sucker consists of 6-10 (average, 9) pairs of radiating muscles while the posterior sucker has 7-14 (average, 11) pairs. Next to the posterior sucker and ending a short distance in front of the cloaca are 12-18 (average, 14) pairs of oblique muscles. The posterior extremity is strongly curved toward the venter and narrows in diameter just posterior to the cloaca, terminating in a short tail 0.25-0.35 mm long (Figure 4). There are 10 pairs of caudal papillae and a prominent single adcloacal papilla. Of the paired papillae, 5 pairs are preanal and of these; the first three pairs are

lateral and the last two pairs are ventrolateral. All five pairs of postanal papillae are ventral except the second and third pairs which are lateral (Figure 5).

Both spicules are equal, curved, alated and laterally compressed. They contain a tubular central portion and measure 0.52-0.91 mm (average, 0.65mm) long and 0.39-0.76 mm (average, 0.70 mm) wide. In some specimens, the spicules bear spine-like alar supports along the margin. The saddle-shaped accessory piece is prominent in some specimens but weakly developed in others. It measures 0.09-0.15 mm (average, 0.13 mm) long and lies posterior to the spicules (Figure 4).

Female (n = 89)

Females are 6.9-11.0 mm (average, 9.0 mm) long. They are 0.42-0.47 mm (average, 0.44 mm) wide at the level of the esophagus, 0.49-0.64 mm (average, 0.56 mm) wide at the vulva, 0.09-0.47 mm (average, 0.28 mm) wide at the level of the anus and narrows progressively towards the posterior to end in a whip-like tail. The tail is 0.85-1.10 mm (average, 0.97 mm) long. The buccal cavity is 0.03-0.04 (average, 0.03 mm) long and 0.09-0.16 mm (average, 0.13 mm) wide; and the pharynx is 0.04-0.05 mm (average, 0.04 mm) long and 0.09-0.18 mm (average, 0.15 mm) wide. The cylindrical esophagus extends for 0.86-1.24 mm (average, 1.19 mm) at the width of 0.15-0.23 mm (average, 0.18 mm) before reducing to 0.11-0.14 mm

(average, 0.12 mm) wide for the remaining 0.17-0.20 mm (average, 0.18 mm) of its length. The prebulb is 0.9-0.15 mm (average, 0.12 mm) long and 0.14-0.21 mm (average, 0.16 mm) wide and the larger postbulb measures 0.29-0.34 mm (average, 0.30 mm) long by 0.26-0.32 mm (average, 0.29 mm) wide. The nerve ring is 0.13-0.42 mm (average, 0.32 mm) from the cephalic end.

The vulvar opening is guarded by two ill-defined lips and is situated on the ventral side 5.4-6.4 mm (average, 5.7 mm) from the anterior end of the body. From the vulva, the muscular vagina runs dorso-cephalad for 0.56-1.05 mm (average, 0.75 mm) before dividing into two opposed uteri. The cephalad uterus runs forward, doubling back and forth on itself, and forms an elongated fold lateral to the anterior end of the intestine before connecting to the anterior ovary. The caudad uterus, after a course in this direction, turns cephalad to form another elongated fold opposite that of the former before turning caudally again to connect to the posterior ovary. The complete course of the uteri is obscure as they are laden with large oval granular eggs, measuring 0.09-0.18 mm (average, 0.14 mm) long and 0.09-0.25 mm (average 0.21 mm) wide (Figure 6).

The posterior extremity is slightly curved and the tail is nearly straight (Figure 3).

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Figure I Spironoura duyagi, anterior end -- showing labial, esophageal and anterior intestinal morphology.

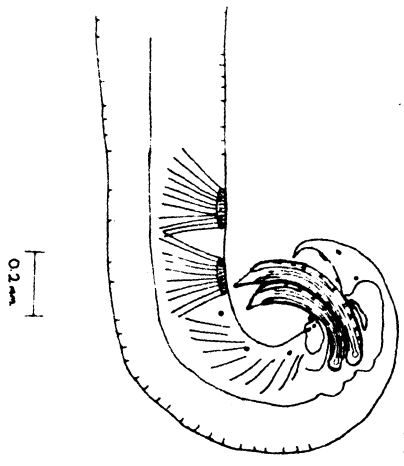
Figure II Enface view -- showing cephalic morphology.

Figure III Posterior extremity of female -- lateral view.

Figure IV Posterior extremity of male, showing oblique muscles spicules and accessory piece -- lateral view.

Figure V Posterior extremity of male, showing pseudosuckers and positions of caudal papillae -- ventral view.

Figure VI Section of female at region of vulva -- lateral view.



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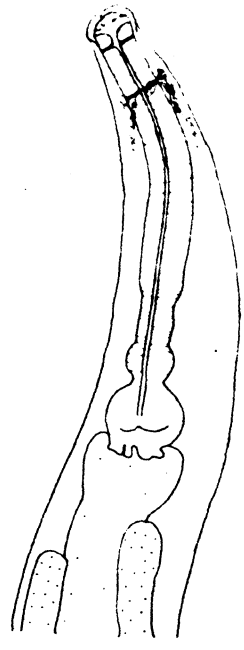

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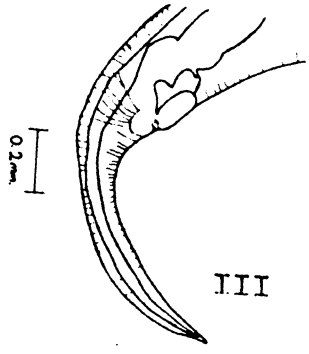

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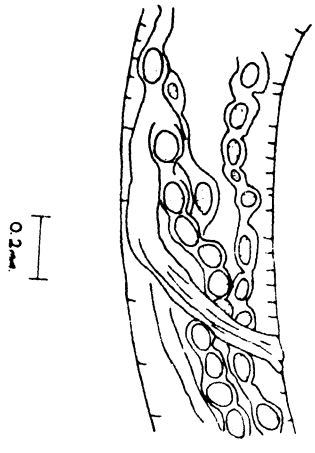

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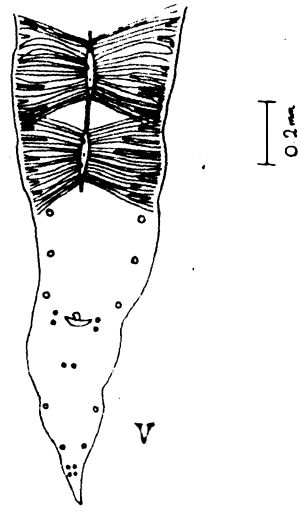

V

DISCUSSION

There can be no doubt that the worms under consideration conform to the description given by Leidy (1856) for genus Spironoura. They also are similar to that given by Lane in 1915 (Baylis and Daubney, 1922) for the genus Falcaustra. The characters for both genera are very similar and it appears that Falcaustra is a mere amplification of Spironoura. Yorke and Maplestone [1926] 1962, apparently failed to see any difference between the two and very reasonably regarded Falcaustra synonymous to Spironoura.

Compared to some spironouran species described from fish hosts, the absence of a pseudosucker in the males of F. leptocephala (Baylis and Daubney, 1922); S. congolense (Taylor, 1925), S. nilgiriensis (Soota and Chaturvedi, 1971); and F. therenzieni (Petter, 1979) readily distinguishes these four species from the present specimens. However, the present specimens from Cuora amboinensis show affinity to S. petrei (Khalil, 1970) and F. tchadi (Vassiliades and Troncy, 1973) by having cephalic semicircular structures that look very much like amphids; but, the double pseudosuckers present in the ventral caudal extremity of the males differentiate them from the latter two species and also from F. barbi (Baylis and Daubney, 1922), S. hexapapillata (Khalil, 1962), and S. sudanensis (Khalil, 1962), each of which have only one pseudosucker.

Turning to the several spironourans of amphibians and

reptilians other than turtles; the worms in question differ from Florencioia mascula (Travassos, 1920); F. nitida (Travassos, 1920); S. elongata (Walton, 1932); Falcaustra golvani (Chabaud and Brygoo, 1957); F. chabuadi (Dyer, 1973) by having more than one pseudosucker in the male. They further differ from S. elongata (Walton, 1932) by the absence of a culticularized tooth in the inner surface of the labia; and from F. chabaudi (Dyer, 1973) by the absence of an elongated isthmus between the two esophageal bulbs. The absence of a description of a male in the report of S. crytobranchi (Walton, 1930) does not allow a meaningful comparison with the present species but the larger body of the former is a point of difference. The extremely short body (3.5-4.5 mm) of S. catesbianae (Walton, 1929) contrasts with that of this species, while the absence of a pseudosucker in the males of S. gracile (Leidy, 1858), S. brevispiculata (Baylis, 1935) and S. trilokiae (Singh, 1958; Khalil, 1962) differentiates them from the present species.

The type specimen of Velariocephalus trilokiae (Singh, 1958) was not accessible for examination but after a study of the description and diagrams given by Singh (1958) for the genus and species, I agree with Khalil (1962) in regarding Velariocephalus synonymous to Spironoura.

The fact that S. cameroni (Jehan, 1970a) is found to occur in Coturnix coturnix makes it rather "alien" to the other spironourans, and as far as it could be determined from published literature, it is

the only spironouran species so far reported from an avian host. Coturnix coturnix is probably an accidental host.

Of the species described from turtle or tortoise hosts, the absence of a pseudosucker in the male of Falsaustra chapini (Boulenger, 1923 = S. affine; Leidy, 1856; Mackin, 1936), F. falcata (Lane, 1915); F. lambdiensis (Seurat, 1918); F. longispicula (Walton, 1927); F. stewarti (Baylis, 1933; Baylis and Daubney, 1922; Jehan, 1970b), S. elseyae (Johnston and Mawson, 1941); S. fernandoi (Sathananthan, 1972), S. gracile (Leidy, 1856); S. kachugae (= oxysoma kachugae) (Steward, 1914); S. procera (Canavan, 1929); and S. tikasinghi (Schoenecker et al, 1977) differentiates them from the specimens in this study. But these present specimens further differ from S. chelydrae (Mackin, 1936); S. concinnae (Mackin, 1936); S. intermedia (Caballero, 1939); S. onama (Karve, 1927); S. rangoonica (Chatterji, 1936); and S. wardi (Mackin, 1936) by possessing more than one pseudosuckers, and from S. siamensis (Baylis, 1920; 1933) and S. pillaii (Sathananthan, 1972) in having less than three pseudosuckers.

Bearing lips that project in front of the head furthers the relation of the specimens in this study with F. chapini (Boulenger, 1923) and S. concinnae (Mackin, 1936), while the heavily pigmented arcade cells further identify them with S. concinnae (Mackin, 1936) and also with S. wardi (Mackin, 1936) and S. affine (Mackin, 1936) but they further differ from the former two species in body size and from the latter three in that the arcade cells extend beyond the nerve ring. The

present specimens also contrast with S. japonensis (Yamaguti, 1935) in that none of the males examined possess less than two pseudo-suckers.

Tubangui and Villaamil (1933) describe Spironoura duyagi from the caecum of Cyclemis amboinensis. This host species is synonymous to Cuora amboinensis from which the specimens of the present investigation are obtained. At first sight, it is hard to differentiate the morphology of the specimens in this study from the description given by Tubangui and Villaamil (1933) for S. duyagi. For among other commonalities, both have similar lip structure, identical number and arrangement of caudal papillae, and similar esophagus. But a close study of the two shows that the original description of S. duyagi (Tubangui and Villaamil, 1933) is a performance that warrants a reassessment.

The authors state the average measurements of the males and females of S. duyagi as 11.5-13.0 mm long by 0.40-0.72 mm wide and 13.0-15.0 mm long by 0.78-1.00 mm wide respectively. They make no reference to the number of specimens examined or the number of turtles from which the specimens are collected. As they stand, these figures contrast strongly with those of the present investigation which are 6.00-9.7 mm long by 0.44-0.51 mm wide and 6.9 -11.0 mm long by 0.49-0.64 mm wide. The probabilities are either a very limited sample is involved in the 1933 investigation or the results are based on a sample of selected specimens. Distortion, that could be

caused by the preservation of the specimens is also a factor. The wider range in measurements shown in the present investigation express a more accurate picture of the variations that exist within the species and the fact that 226 worms from three turtles are involved is evidence that a better representation of the species is presented.

The description of the species by Tubangui and Villaamil is not without confusion. The authors state that the male of S. duyagi generally has two genital suckers (pseudosuckers) with occasionally an extra one, but show no more than one of these structures in the diagramatic representation of the species. They also refer to the accessory genital structure as gubernaculum but label it "accessory piece" at the same time giving it the features of a gubernaculum in one of the diagrams.

Of the 226 specimens, none of the 137 male worms examined in the present investigation, was found to possess more than two genital suckers. Traces of an excretory pore and cervical papillae mentioned by the authors for S. duyagi are not visually evident in the present specimens. Furthermore, the authors fail to mention anything about the arcade cells that form such a unique feature around the nerve ring and appear so distinctly in the present samples. They also say nothing about the faint semi-circular areas that lie lateral to the stomodial lips.

Yorke and Maplestone ([1926] 1962) list 16 species in the genus Spironoura. Yamaguti (1961) records about 45 different species and

groups them according to their reptilian, amphibian and fish hosts. The period from 1961 to present has seen the discovery of several new species which should be added to Yamaguti's list:

S. cameroni (Jehan, 1970a) in Coturnix coturnix

S. chabaudi (Dyer, 1973), syn. Falcaustra chabaudi, in Siren intermedia nettingi

S. fernandoi (Sathananthan, 1972) in Geomyda trijuga thermalis

S. hexapapillata (Khalil, 1962) in Distichodus niloticus

S. nilgiriensis (Soota and Chaturvedi, 1971) in Puntius cornaticus

S. petrei (Khalil, 1970) in Distichodus rostratus

S. pillaii (Santhananthan, 1972) in Geomyda trijuga thermalis

S. sudanensis (Khalil, 1962) in Distichodus brevipennis

S. tchadi (Vassiliades and Troncy, 1973) syn. Falcaustra tchadi in Distichodus brevipinnis

S. therezieni (Petter, 1979) syn. Falcaustra therezieni in Ptychochromoides betsileanus and Arius madagascariensis

S. tikasinghi (Schoenecker et al., 1977) in Geomyda punctularia

S. trilokiae (Khalil, 1962) syn. Velariocephalus trilokiae (Singh, 1958) in Rana cyanophlyctis

I am also aware of the existence of S. straeleni (Campana-Rouget, 1961); S. verbekei (Campana-Rouget, 1961) and S. fordoniae (Jones, 1979) but published literature on these species is unavailable to me.

The fact that the present specimens are smaller, bear well defined arcade cells, possess amphid-like structures that lie lateral to the stomodial lips, and have no more than two pseudosuckers in the male; leads me to believe that a further study will demonstrate that these worms are a different species to S. duyagi.

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A REDESCRIPTION OF SPIRONOURA DUYAGI FROM THE STOMACH OF MALAYAN BOX TURTLES (CUORA AMBOINENSIS)

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ABSTRACT OF A THESIS

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ABSTRACT

Two hundred and twenty-six worms taken from the stomachs of three Malayan box turtles (Cuora amboinensis) were studied. The worms were identified as Spironoura spp. and their numerical measurements and morphological details compared to other described species in the genus. The nematodes, generally, conformed to published descriptions of Spironoura duyagi, but varied so widely with regards to presence or absence of key diagnostic features that a complete taxonomic analysis was undertaken.