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**EVALUATION OF PARASITE MARKERS TO ACCESS SWORDFISH STOCK
STRUCTURE**

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Evaluation of Parasite Markers to Assess Swordfish Stock Structure

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Executive Summary

A preliminary trial was undertaken to determine if there are appropriate parasites in broadbill swordfish (*Xiphias gladius*) for testing residency hypotheses and stock relationships. The gills and guts of 34 swordfish, eight from New Caledonia, 10 from Australia (Queensland), and 16 from New Zealand, were examined for parasites. Three species of monogenean were found on the gills (*Tristoma adintegrum*, *Tristoma adcoecineum* and an unidentified capsalid); three species of nematode in the stomach (*Maricostula* sp., *Hysterothylacium* sp. A, and *Hysterothylacium* sp. B); two cestodes in the stomach and encysted in the mesenteries (*Pseudeubothrium* sp. and *Hepatoxylon* sp.); and one digenean in the stomach (*Hirudinella* sp.). Swordfish from New Caledonia were also examined for ectoparasites.

The parasite fauna of swordfish from the three areas was dominated by adult nematodes. The largest of the nematodes, *Maricostula* sp., showed differences in abundance among the three areas. The other two nematodes, species of *Hysterothylacium*, also showed significant differences between areas, but these adult worms may have limited application as a biological tag, reflecting short-term feeding patterns of the host. Three parasites were identified that are potential markers of movement of swordfish between tropical and temperate waters. 1) The digenean *Hirudinella* is likely to be a short lived parasite acquired in tropical areas. 2) Larval cestodes of *Hepatoxylon* sp. are thought to be acquired in temperate areas. 3) A pennellid copepod recorded in swordfish from New Caledonia, is likely to be acquired in tropical areas. This large ectoparasite is readily observed on whole swordfish, and presence/absence could be recorded by fishery observers, without the need for returning samples to the laboratory.

Introduction

Experience in the Mediterranean, Atlantic and North Pacific has shown swordfish (*Xiphias gladius*) stocks to be susceptible to overfishing. Commercial catches of swordfish in New Zealand and Australian waters increased rapidly in the 1990's, when catches on the high seas between the two countries expanded. However, catches have declined in recent years. Research is underway to explore CPUE in New Zealand fisheries and the commercial swordfish catch is monitored by at-sea observers and port sampling programmes. Observers collect data on the size structure of the commercial catch to allow monitoring of trends in the size structure of the commercial catch of swordfish.

Recent genetic research using mitochondrial DNA markers has suggested that there is a separate South West Pacific stock of swordfish (Reeb et al. 2000; Lu *et al.*, 2006; Ward et al., 2001), but very little is known of the movement of swordfish within this region. An understanding of sub-stock structure in the South West Pacific region is required if this species is to be effectively managed. Knowledge of movement patterns would also contribute to more effective modelling of the stocks in the region. This investigation was undertaken to evaluate the potential of parasite markers as a tool to determine stock relationships of swordfish in the New Zealand EEZ and wider South West Pacific Ocean.

Methods

The gills and guts from swordfish were collected in three areas of the Southwest Pacific Ocean. Samples were collected by Observers on commercial fishing vessels and frozen before freighting to Wellington. The gills and guts were collected from 8 swordfish in New Caledonia (mean length 128 cm, range 66 – 292 cm), 10 from Australia (Queensland) (mean length 131 cm, range 106 – 174 cm), and 16 from New Zealand (mean length 216 cm, range 120 – 258 cm). Samples from New Caledonia were supplied by Valerie Allain (Secretariat of the Pacific Community); from Queensland by Martin Scott (Australian Fisheries Management Authority Observer Programme); and from New Zealand by the Ministry of Fisheries Observer Programme.

In the laboratory frozen gills and guts were thawed and dissected. Parasites in the gills and guts were located using a dissecting microscope and placed in 10% formalin for later identification using a compound microscope. The types and numbers of parasites present were recorded for each swordfish.

The ecological terminology used to describe the distribution of parasites amongst the swordfish samples followed that recommended for marine fishes (Bush et al., 1997):

prevalence = number of infected fish divided by number of fish examined, expressed as a percentage, and

intensity = mean number of parasites found among the infected fish.

The criteria used to determine whether a parasite had potential for use as a stock discriminator followed those established for marine fishes (Lester, 1990; MacKenzie, 1987):

- the parasite should have a lifespan, or remain in identifiable form, in the host long enough to cover the time scale of the investigation;
- the parasite should occur at a reasonably high prevalence; and
- the parasite should be easily detected and identified.

Between-area differences in parasite abundance were tested with randomisation tests; firstly employing only positive counts, and secondly, employing all observations, including 0 counts. A total of 500 bootstrapped datasets were generated for each species of parasite. Two-sided *P*-values were calculated by comparing the observed differences in mean abundance to the bootstrap distributions (Tables 3A and 4A). To control for the length of the swordfish, linear regressions of parasite numbers on length were performed, and the residuals from these regressions used in the randomisation tests (Tables 3B and 4B).

Results

The parasites found in the swordfish samples included three species of monogenean on the gills (*Tristoma adintegrum*, *Tristoma adcooccineum* and an unidentified capsalid, see Figures 1-5); three species of nematode in the stomach (*Maricostula* sp., *Hysterothylacium* sp. A, and *Hysterothylacium* sp. B, see Figure 6); two cestodes, one in the stomach (*Pseudeubothrium* sp.) and one encysted in the mesenteries (*Hepatoxylon* sp.); and one digenean in the stomach (*Hirudinella* sp., Figure 7). Swordfish from New Caledonia had been examined for ectoparasites by fishery observers, and three species of parasitic copepods, *Gloiopotes* sp., *Caligus* sp., and a member of the family Penellidae (Figure 8), were recorded.

The predominant groups of parasites found in the swordfish were helminths, namely Monogenea (two species of *Tristoma*), Nematoda (*Maricostula* sp. and *Hysterothylacium* spp.), and Cestoda (Tables 1 and 2). Similar findings were reported for parasites in swordfish sampled in the Atlantic Ocean (DiPaolo et al., 1994; Hogans et al., 1983; Williams & Bunkley-Williams, 1996). Helminths were also the predominant parasite group in black marlin collected off the east coast of Australia (Speare, 1994).

Table 1: Prevalence of parasites (%) recovered from swordfish collected from three regions of the Southwest Pacific. n/a = not available.

	New Caledonia	Queensland	New Zealand
Number of fish examined	8	10	16
Mean fork length (cm)	128.25	131	216
Range (cm)	66-292	106-174	120-258
Monogenea			
<i>Tristoma adintegrum</i>	87.5	50	62.5
<i>Tristoma adcooccineum</i>	50	20	31.25
Capsalid	0	0	6.25
Crustacea			
<i>Gloiopotes</i> sp.	25	n/a	n/a
<i>Caligus</i> sp.	12.5	n/a	n/a
Pennellid copepod	12.5	n/a	n/a
Nematoda			
<i>Maricostula</i> sp.	37.5	50	56.25
<i>Hysterothylacium</i> sp. A	50	80	43.75
<i>Hysterothylacium</i> sp. B	12.5	30	87.5
Cestoda			
<i>Pseudeubothrium</i> sp.	25	n/a	n/a
<i>Hepatoxylon</i> sp.	12.5	10	18.75
Digenea			
<i>Hirudinella</i> sp.	0	0	6.25

Numerically the parasite fauna of swordfish from all three areas of the Southwest Pacific was dominated by adult nematodes (*Maricostula* sp. and *Hysterothylacium* spp., Figure 6) found in the stomach. The largest of these parasites, *Maricostula* sp., were up to 16 cm long and easily identified. *Maricostula* sp. are thought to live for several years (Hogans & Bratney, 1982), making them suitable biological tags. There were significant area differences (Tables 3A and 4A), but these differences were influenced by host length (Tables 3B and 4B). The New Zealand swordfish were mostly large (mean length 216 cm) and *Maricostula* sp. were not present in small fish (<180 cm). In contrast the New Caledonia fish were mostly small (mean length 128 cm) and *Maricostula* sp. were only present in the larger fish (>174 cm). *Maricostula* sp. showed low intensities in the small swordfish (mean 131, maximum 174 cm) from Queensland.

At least two species of *Hysterothylacium* were present in swordfish from all three areas, including very heavy infestations in some of the larger swordfish (Table 2). *Hysterothylacium* sp. A was prevalent in the stomachs of swordfish from Queensland (Table 1) and occurred at very high intensities in swordfish from New Caledonia

(Table 2), noticeably the 3 largest swordfish (>174 cm). There were significant differences in abundance of *Hysterothylacium* sp. A in swordfish from New Caledonia and New Zealand, and from New Caledonia and Queensland, and these differences were not affected by host length (Tables 3 and 4).

Hysterothylacium sp. B occurred at high intensities in swordfish from New Zealand (Tables 1 and 2), but at low intensities (<5) in the smaller swordfish (<180 cm). Only one swordfish from New Caledonia was infested with *Hysterothylacium* sp. B, and this was the single large specimen (292 cm). Swordfish from Queensland had low intensities of *Hysterothylacium* sp. B. There were significant area differences between New Zealand and New Caledonia, and between New Zealand and Queensland, but these differences were influenced by host length (Table 4).

The *Hysterothylacium* spp. were observed as adult worms, which have a lifespan much less than that of larval *Hysterothylacium* (which can survive for many years encysted in the mesenteries). In swordfish in the Atlantic Ocean some infections of adult *Hysterothylacium* sp. are accidental infections temporarily acquired from prey (Hogans et al., 1983). Thus adult *Hysterothylacium* sp. may have limited application as a biological tag, reflecting short-term feeding patterns. Furthermore definitive identification of these worms can be difficult, with genetic results revealing the presence of cryptic species in morphologically indistinguishable *Hysterothylacium* from Atlantic swordfish (Mattiucci et al., 1994).

Swordfish from New Caledonia had the highest prevalences and intensities of both *Tristoma* species (Tables 1 and 2). *Tristoma adintegrum* showed significant area differences (Tables 3 and 4) with the lowest prevalences and intensities in swordfish from Queensland (Tables 1 and 2). There were no area differences for *Tristoma adcoecineum* (Tables 3 and 4).

The capsalid monogenean and the digenean *Hirudinella* sp. were each found in one swordfish from New Zealand (Table 1). *Hirudinella ventricosa* in skipjack tuna were considered to be a short lived parasite acquired in tropical areas (Lester, Barnes & Habib, 1985). It is possible that the *Hirudinella* sp, found in one swordfish from New Zealand (a large fish, 257 cm), indicated a recent movement of this fish into temperate waters, but this would need to be confirmed by examining larger sample sizes of swordfish.

The cestode *Pseudeubothrium* sp. was found in the intestines of fish from New Caledonia. It was not possible to determine whether this parasite was present in swordfish from New Zealand and Queensland, because the samples were supplied as stomach only, and did not include the intestine or remainder of the gastrointestinal tract.

Larval *Hepatoxylon* sp. are potentially valuable tags as they are long lived. *Hepatoxylon* sp. were recorded at low prevalences and intensities in all three areas (Tables 1 and 2), but there was a significant area difference with the randomisation tests (Tables 3 and 4). These differences were based on a small number of observations and need to be confirmed in larger sample sizes. In albacore tuna *Thunnus alalunga*, the cestode *Hepatoxylon* sp. was considered to be acquired in temperate areas and lost as albacore moved into the tropics (Jones, 1991). The one swordfish in New Caledonia with *Hepatoxylon* sp. was the only large fish (292 cm) in the sample (other fish 66-190 cm), and might have moved from temperate waters.

However, the one swordfish in Queensland waters with larval *Hepatoxylon* sp. was small (123 cm), and was unlikely to have migrated from temperate waters.

The parasitic copepods were not tested as markers because they were only available in samples from New Caledonia (and at low prevalence, Table 2). Pennellid copepods (Figure 8) are one of the few ectoparasites which are long lived parasites and well anchored on the host, and hence unlikely to be lost during capture and handling (Hogans, 1986). Because pennellid copepods are well anchored, and easy to see with the naked eye (embedded on the head, flanks, and fins), they are suitable biological tags for swordfish (Castro-Pampillon et al., 2002; Maksimov, 1970). It is likely that the pennellid copepods are acquired in tropical areas, because these species are usually found on large tropical pelagic fishes (Williams *et al.*, 1996). Observers could record the presence/absence of pennellid copepods, without the need for returning tissue samples or specimens to the laboratory.

Two species of pennellid copepod occur on swordfish in the Atlantic ocean - *Pennella filosa* and *Pennella instructa* (Castro-Pampillon et al., 2002), although both copepods have never been recorded on one host (Williams *et al.*, 1996). The pennellid found on the swordfish from New Caledonia is most likely *P. instructa*, as this species has been previously recorded from billfish in the Southwest Pacific (Speare, 1994, 1999).

The other two copepods *Gloiopotes* sp. and *Caligus* sp., are likely to fall off post capture (Williams *et al.*, 1996), and are not useful stock markers.

Table 2: Mean intensity of parasites recovered from swordfish. n/a = not available

	New Caledonia	Queensland	New Zealand
Monogenea			
<i>Tristoma adintegrum</i>	3	1.2	2.8
<i>Tristoma adcoecineum</i>	1.8	1	1.6
Capsalid	-	-	1
Crustacea			
<i>Gloiopotes</i> sp.	7.5	n/a	n/a
<i>Caligus</i> sp.	1	n/a	n/a
Pennellid copepod	2	n/a	n/a
Nematoda			
<i>Maricostula</i> sp.	29	7.4	111.4
<i>Hysterothylacium</i> sp. A	703.8	11.9	81.4
<i>Hysterothylacium</i> sp. B	100	5.7	372.3
Cestoda			
<i>Pseudeubothrium</i> sp.	4	n/a	n/a
<i>Hepatoxylon</i> sp.	2	1	1
Digenea			
<i>Hirudinella</i> sp.	-	-	2

Table 3: *P*-values from the randomisation tests on swordfish parasites. Values less than 0.05 (shown in bold) identify a statistically significant difference between areas. These results are based on tests of positive (i.e. non- zero) results only. The probability of getting 3 or more significant test results at the 1% level, out of 18 tests is 0.0007 (assuming independence). NC = New Caledonia; NZ = New Zealand, QL = Queensland. NA = not applicable

A. Area, all data	<i>Tristoma. adintegrum</i>	<i>T. adcoecineum</i>	Capsalid	<i>Maricostula</i> sp.	<i>Hysterothyla cium</i> sp.A	<i>Hysterothyla cium</i> sp.B	<i>Hepatoxylon</i> .sp.	<i>Hirudinella</i> sp.
NC-NZ	0.818	0.666	NA	0.128	0.008	0.516	<0.01	NA
QL-NZ	0.058	0.344	NA	0.008	0.722	0.082	0.806	NA
NC-QL	0.038	0.198	NA	0.736	<0.01	0.822	<0.01	NA
B. Area & length								
NC-NZ	0.978	0.452	NA	0.116	<0.01	0.156	<0.01	NA
QL-NZ	0.028	0.692	NA	0.456	0.504	0.766	0.198	NA
NC-QL	0.064	0.268	NA	0.368	<0.01	0.288	0.594	NA

Table 4: *P*-values from the randomisation tests on swordfish parasites. Values less than 0.05 (shown in bold) identify a statistically significant difference between areas. These results include zero results. The probability of getting 3 or more significant test results at the 1% level, out of 18 tests is 0.0007 (assuming independence). NC = New Caledonia; NZ = New Zealand, QL = Queensland. NA = not applicable

A. Area, all data	<i>Tristoma. adintegrum</i>	<i>T. adcoecineum</i>	Capsalid	<i>Maricostula</i> sp.	<i>Hysterothyla cium</i> sp.A	<i>Hysterothyla cium</i> p.B	<i>Hepatoxylon</i> .sp.	<i>Hirudinella</i> sp.
NC-NZ	0.764	0.698	NA	<0.01	<0.01	0.028	<0.01	NA
QL-NZ	0.024	0.098	NA	<0.01	0.518	<0.01	1	NA
NC-QL	0.02	0.062	NA	0.522	<0.01	0.562	<0.01	NA
B. Area & length								
NC-NZ	0.202	0.308	NA	0.306	<0.01	0.066	0.252	NA
QL-NZ	0.102	0.468	NA	0.15	0.668	0.052	0.744	NA
NC-QL	<0.01	0.1	NA	0.78	0.002	0.912	0.39	NA

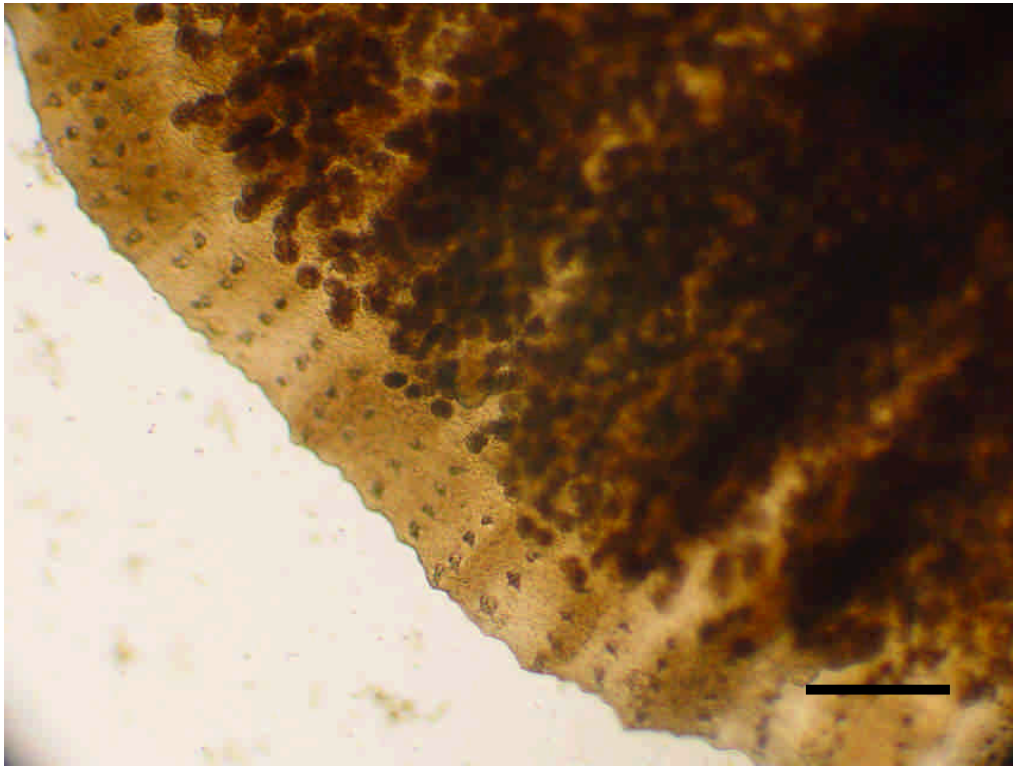


Figure 1: Large numbers of closely spaced marginal hook rows of *Tristoma adintegrum* from swordfish. Scale bar = 350 μm .

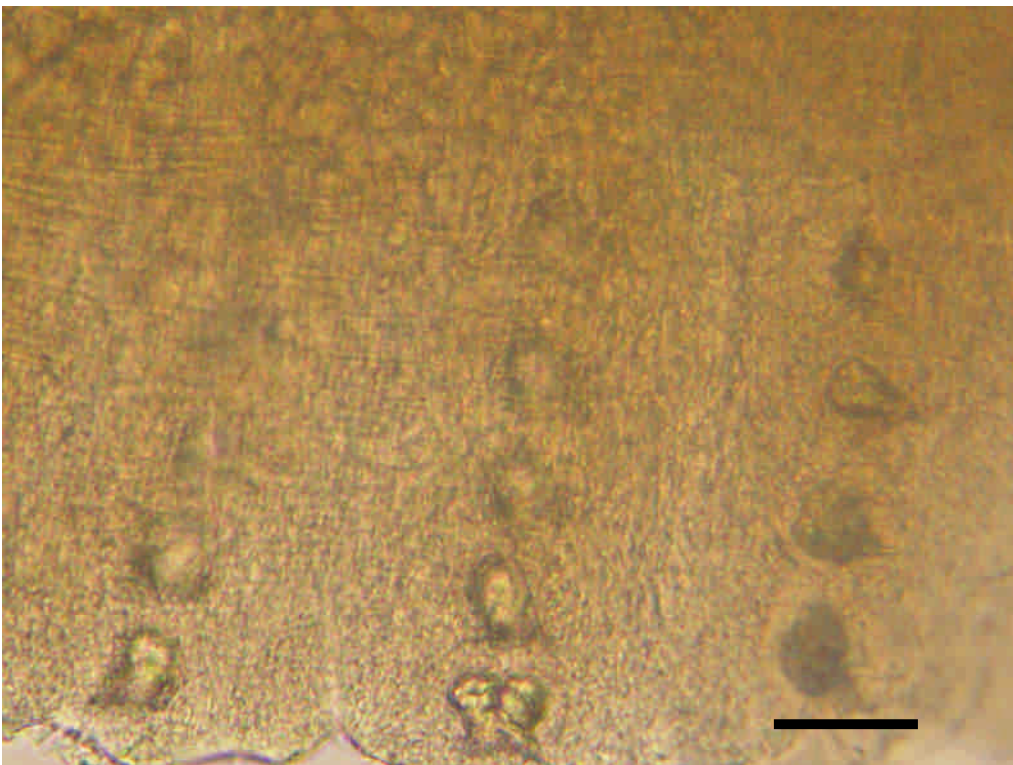


Figure 2: High power view of marginal hook rows of *Tristoma adintegrum* from swordfish. Scale bar = 35 μm .

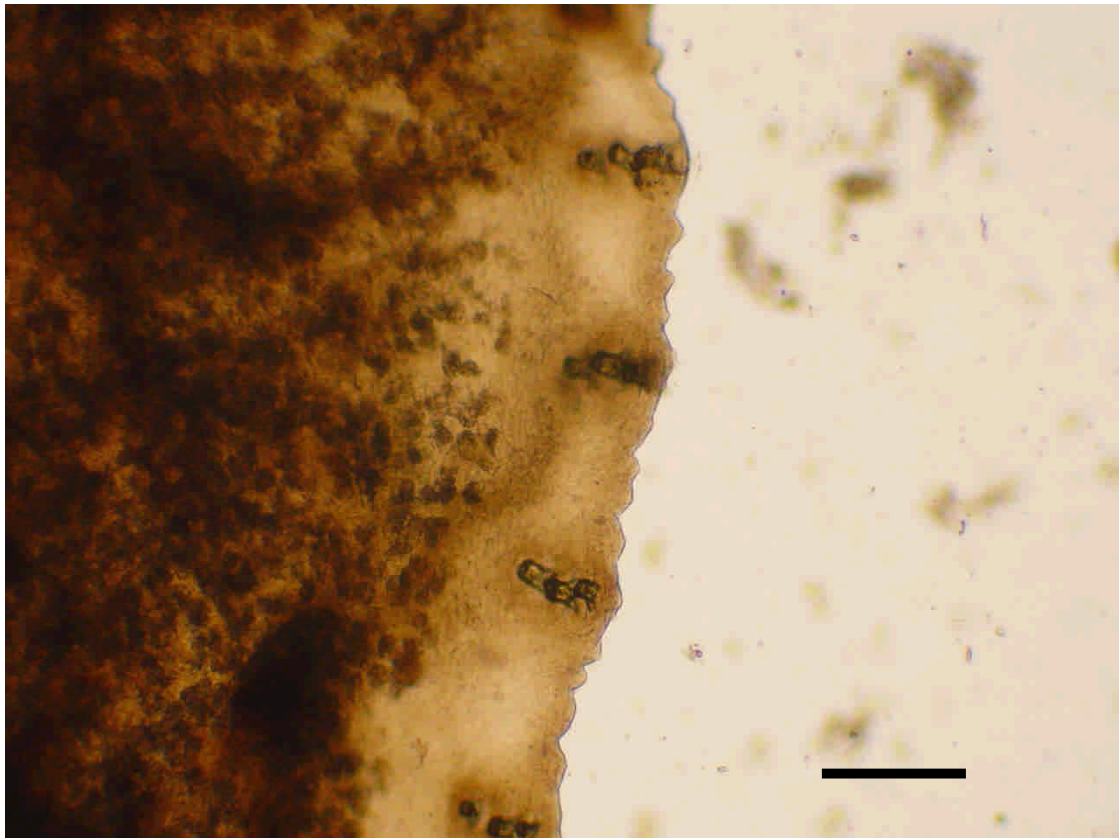


Figure 3: Widely spaced marginal hook rows of *Tristoma adcoccineum* from swordfish. Scale bar= 350 μ m.

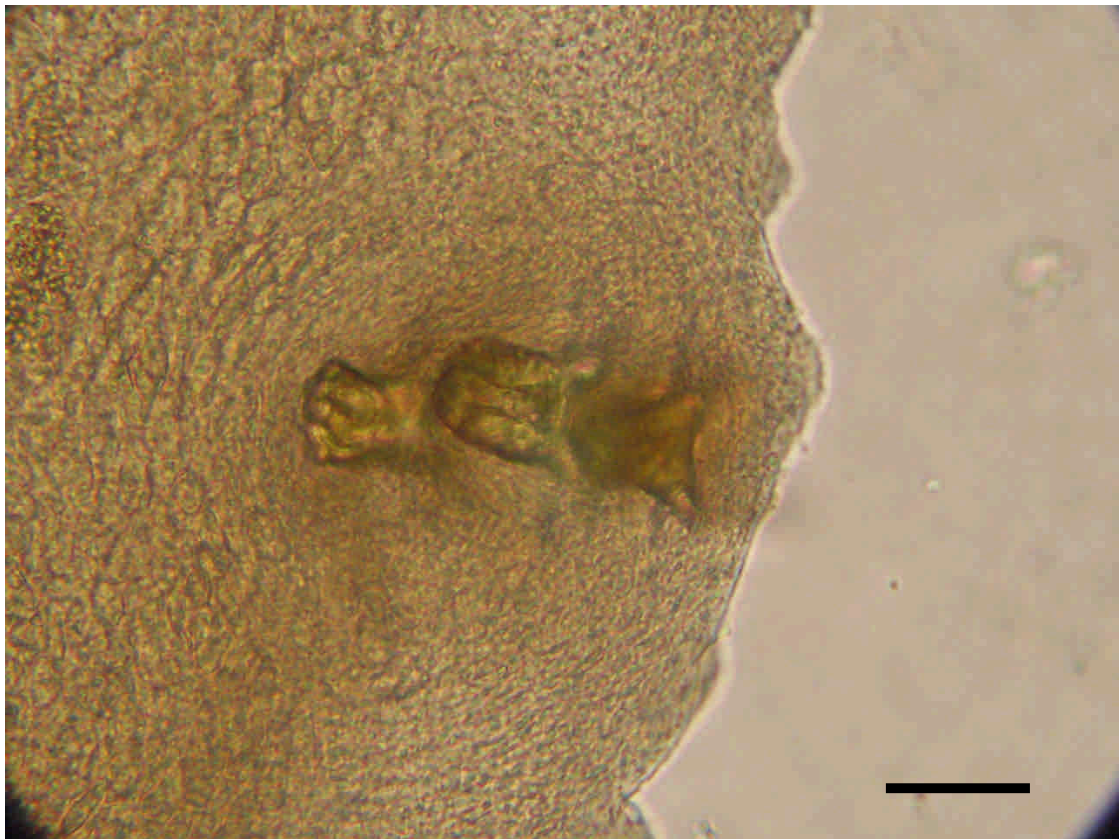


Figure 4: High power view of marginal hook rows of *Tristoma adcoccineum*. Scale bar = 35 μ m.

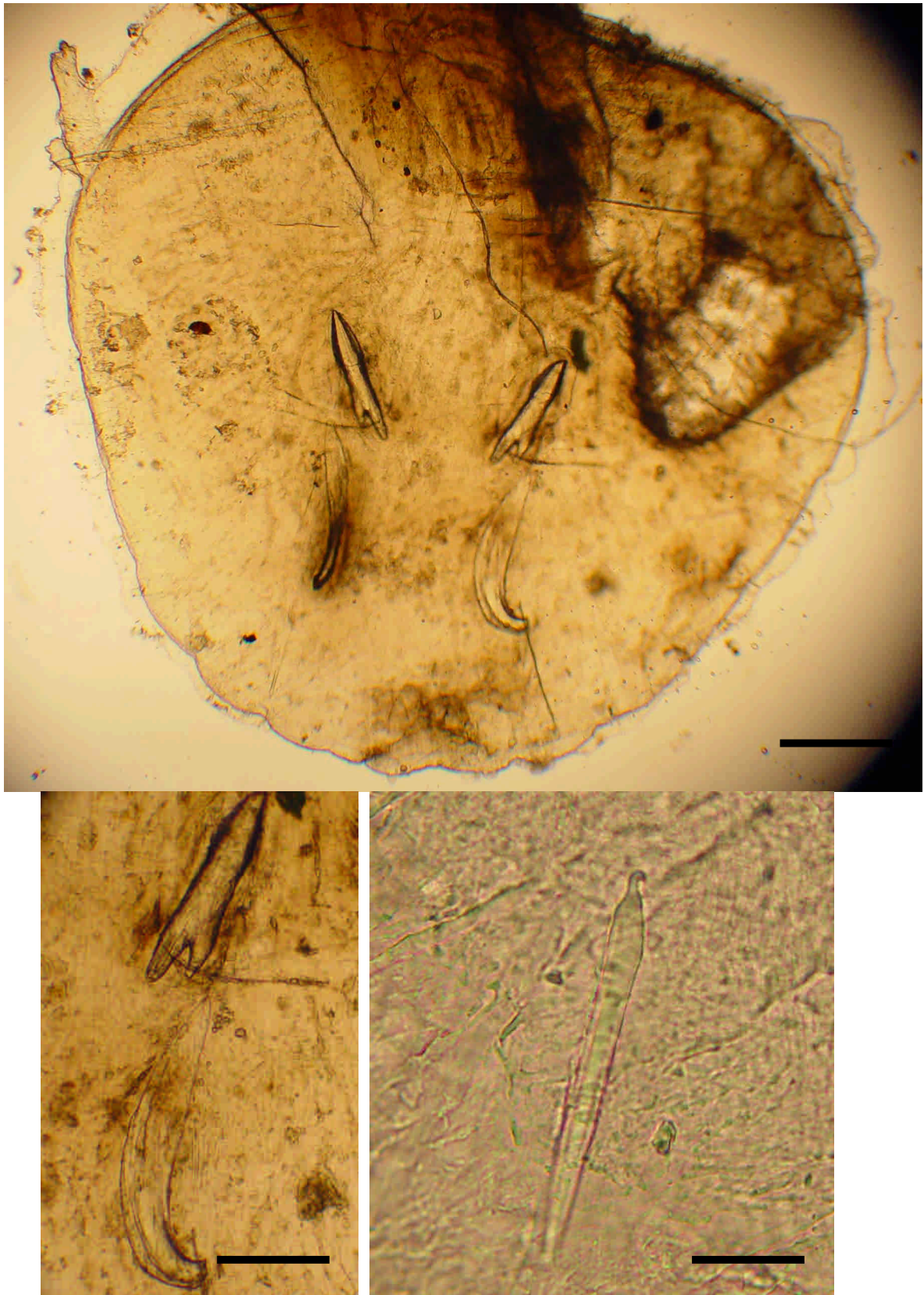


Figure 5: Opisthaptor (top), hamulus (lower left) and marginal hooklet (lower right) of an undescribed capsalid monogenean from the gills of swordfish from New Zealand. Scale bars = 350 μ m, 70 μ m and 35 μ m, respectively.

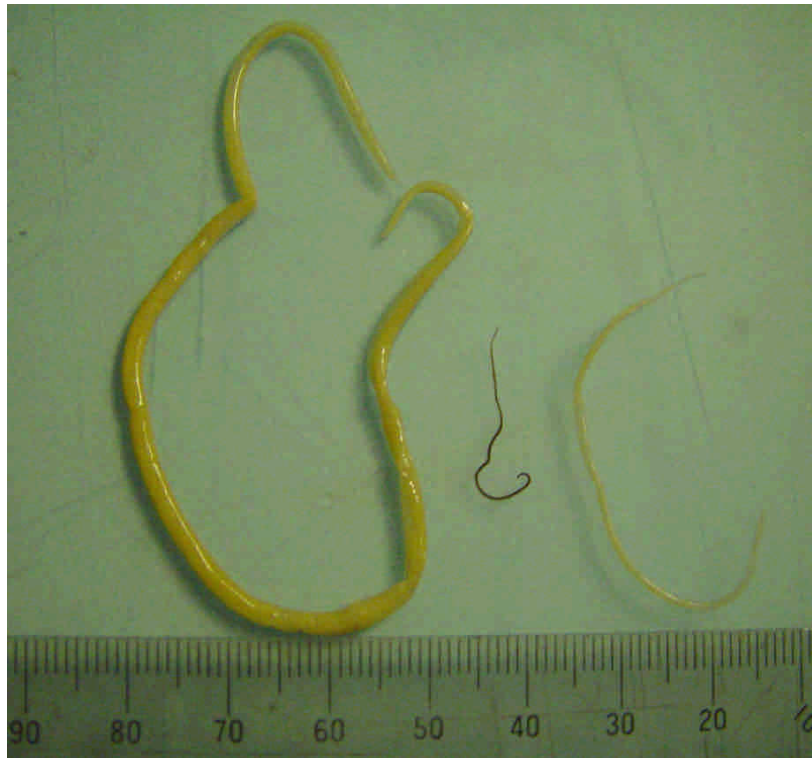


Figure 6: Three nematode species from the stomach of swordfish. From left, *Maricostula* sp., *Hysterothylacium* sp. A, and *Hysterothylacium* sp. B. Scale in mm.



Figure 7: *Hirudinella* sp. from the stomach of swordfish from New Zealand. Scale bar = 500 μ m.



Figure 8: A penellid copepod on swordfish from New Caledonia.

Conclusions

The parasite fauna of swordfish from all three areas of the Southwest Pacific was dominated by adult nematodes, found in the stomach. The largest of these nematodes, *Maricostula* sp., showed differences in abundance among the three fishery areas, but these differences were influenced by host length. Differences between large (<180 cm) swordfish in the tropics and New Zealand EEZ would have to be tested in larger samples. Two other nematodes, both species of *Hysterothylacium*, were present in swordfish from all three areas, and showed significant differences between areas. For *Hysterothylacium* sp. B the area differences were influenced by host length. Both species of *Hysterothylacium* were observed as adult worms, not as larvae, and may represent infections temporarily acquired from prey. Consequently these species may have limited application as a biological tag, reflecting short-term feeding patterns.

Two species of Monogenea were found in swordfish from all three areas. One species, *Tristoma adintegrum* showed significant area differences, which would have to be established in larger sample sizes.

Three parasites were identified that are potential markers of movement of swordfish between tropical and temperate waters. The digenean *Hirudinella* sp. found in one swordfish from New Zealand is likely to be a short lived parasite acquired in tropical areas, and an indicator of recent movement of fish into temperate waters. The presence of this uncommon parasite would have to be tested in larger sample sizes of swordfish.

Larval cestodes of *Hepatoxylon* sp. were recorded at low prevalences and intensities in all three areas. In albacore tuna, the cestode *Hepatoxylon* sp. was thought to be acquired in temperate areas and lost as albacore moved into the tropics. This marker would have to be tested in larger sample sizes of large swordfish to test movement hypotheses between temperate and tropical waters.

A pennellid copepod was recorded by observers in swordfish collected around New Caledonia. These large ectoparasites, are likely to be acquired in tropical areas, and are readily observed on whole swordfish. Their presence/absence could be recorded by fishery observers, without the need for returning tissue samples or specimens to the laboratory for dissection and specialist identification. The application of ectoparasites would have considerable practical and cost advantages over the collection, storage, and freight of frozen gut samples.

It is recommended that a pennellid identification sheet and instructions be prepared for observers to record the presence/absence of this ectoparasite on all swordfish.

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