

Cryptic species complexes in manipulative echinostomatid trematodes: when two become six

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SUMMARY

Recent studies have shown that some digenean trematodes previously identified as single species due to the lack of distinguishing morphological characteristics actually consist of a number of genetically distinct cryptic species. We obtained mitochondrial 16S and nuclear ITS1 sequences for the redial stages of *Acanthoparyphium* sp. and *Curtuteria australis* collected from snails and whelks at various locations around Otago Peninsula, New Zealand. These two echinostomes are well-known host manipulators whose impact extends to the entire intertidal community. Using phylogenetic analyses, we found that *Acanthoparyphium* sp. is actually composed of at least 4 genetically distinct species, and that a cryptic species of *Curtuteria* occurs in addition to *C. australis*. Molecular data obtained for metacercariae dissected from cockle second intermediate hosts matched sequences obtained for *Acanthoparyphium* sp. A and *C. australis* rediae, respectively, but no other species. The various cryptic species of both *Acanthoparyphium* and *Curtuteria* also showed an extremely localized pattern of distribution: some species were either absent or very rare in Otago Harbour, but reached far higher prevalence in nearby sheltered inlets. This small-scale spatial segregation is unexpected as shorebird definitive hosts can disperse trematode eggs across wide geographical areas, which should result in a homogeneous mixing of the species on small geographical scales. Possible explanations for this spatial segregation of the species include sampling artefacts, local adaptation by first intermediate hosts, environmental conditions, and site fidelity of the definitive hosts.

Key words: cryptic species, phylogeny, Echinostomatidae, 16S, ITS1, spatial segregation.

INTRODUCTION

Cryptic species, or genetically distinct lineages that have previously been classified as a single nominal species due to their superficially indistinguishable morphological characteristics, are becoming increasingly recognized as an important issue for the study of ecology and evolutionary biology (Bickford *et al.* 2006). Beyond the obvious implications it holds for obtaining an accurate inventory of existing biodiversity, the failure to recognize cryptic species in medically, economically or ecologically important organisms can have serious negative or costly consequences for the development of biological control measures (Bidochka *et al.* 2001; Walter and Campbell, 2003; Rafter *et al.* 2008), the monitoring and control of human parasites and potential zoonoses (Cepicka *et al.* 2005; Pringle *et al.* 2005; Saijuntha *et al.* 2007), the management of agricultural and aquaculture pathogens (Beauchamp *et al.* 2002; Skobgaard *et al.* 2002), and detecting the

presence of invasive species (Geller *et al.* 1997; Geller, 1999; Miura *et al.* 2006).

In the last few years certain taxa of trematodes that were previously identified as a single species based on morphological features have been shown to consist of many genetically distinct cryptic species (e.g., Donald *et al.* 2004; Miura *et al.* 2005; Saijuntha *et al.* 2007). This coincides with an increase in the use of genetic markers to identify cryptic species complexes and resolve taxonomic questions among parasitic platyhelminths (Vilas *et al.* 2005; Nolan and Cribb, 2005). Given that cryptic species vary in key species-specific traits, and that trematodes have been found to play keystone roles in intertidal ecosystems (Mouritsen and Poulin, 2002) by mediating biodiversity (Mouritsen and Poulin, 2005) and productivity (Wood *et al.* 2007) through altered host phenotype, it is important to explore their true diversity in order to understand the mediating role they can play in intertidal ecosystem functioning.

The New Zealand cockle, *Austrovenus stutchburyi*, serves as the second intermediate host for 2 species of trematodes that encyst in its foot. These two species, *Curtuteria australis* (Allison, 1979) and *Acanthoparyphium* sp. of Martorelli *et al.* (2006), are both from the subfamily Himasthlinae and of the family Echinostomatidae. Due to similarity in their

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Table 1. Sampling site and number of *Curtuteria* spp. and *Acanthoparyphium* spp. samples

Sampling site	<i>Curtuteria</i>		<i>Acanthoparyphium</i>			
	<i>Curtuteria australis</i>	<i>Curtuteria</i> cryptic species	Sp. A	Sp. B	Sp. C	Sp. D
Company Bay	0	0	14	0	0	0
Deborah Bay	1	0	0	0	0	0
Lower Portobello Bay	17	0	17	2	3	0
Turnbull	0	0	13	0	1	0
Hooper's Inlet	1	3	0	0	0	0
Papanui Inlet	2	12	5	0	8	2
Total	21	15	49	2	12	2

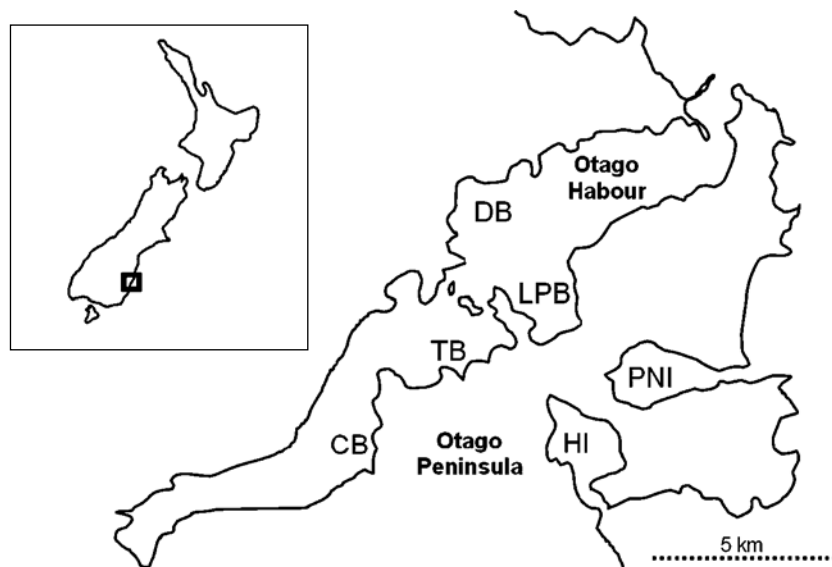


Fig. 1. Map of the study area showing sites where infected hosts were collected. The collection sites are: CB, Company Bay; TB, Turnbull Bay; DB, Deborah Bay; LPB, Lower Portobello Bay; HI, Hooper's Inlet; PNI, Papanui Inlet.

abundance levels and the identical ecological roles they appear to play in the soft-sediment intertidal ecosystem, they are considered to be ecological equivalents (Babirat *et al.* 2004). These two trematodes manipulate their cockle host by impairing its ability to burrow into the sediment (Thomas and Poulin, 1998; Mouritsen, 2002). This not only facilitates their transmission to shorebird definitive hosts, but it also results in marked changes to the structure and diversity of the whole intertidal benthic community (Thomas *et al.* 1998a; Mouritsen and Poulin, 2005). While they utilize the same second intermediate host and share a similar niche within that host, *C. australis* and *Acanthoparyphium* sp. have different first intermediate hosts: the former uses the mud whelk, *Cominella glandiformis* (see Allison, 1979), and the latter the mud snail *Zeacumantus subcarinatus* (see Martorelli *et al.* 2006).

Although the morphologies of both species have been described, here we use phylogenetic analyses of nuclear and mitochondrial genes to reveal that 2 or more genetically distinct lineages exist within both

Acanthoparyphium sp. and *C. australis*. In addition, we uncover a distinct small-scale spatial segregation among the clades within each genus that is totally unexpected given the dispersal potential of avian definitive hosts.

MATERIALS AND METHODS

The parasites for this study were collected over the course of 3 years (Table 1). All hosts and parasites were collected from Otago Harbour and the nearby inlets along Otago Peninsula, South Island, New Zealand. Mud snails, *Zeacumantus subcarinatus*, were collected from Company Bay, Lower Portobello Bay, Turnbull Bay, and Papanui Inlet, while mud whelks, *Cominella glandiformis*, were collected from Deborah Bay, Lower Portobello Bay, Hooper's Inlet, and Papanui Inlet. Cockles, *Austrovenus stutchburyi*, were also collected from Company Bay and Lower Portobello Bay (see Fig. 1). The bivalve *Macomona liliana* was also collected from Company Bay, as it is also known to harbour foot-encysting echinostome

metacercariae (Leung and Poulin, 2008). The animals were all brought back alive to the laboratory and dissected for parasites; *Acanthoparyphium* sp. rediae from *Z. subcarinatus*, *Curtuteria australis* rediae from *C. glandiformis*, and the metacercariae of both species from *A. stutchburyi* and *M. liliana*.

Each redia or metacercaria was carefully isolated from host tissue and transferred into a Petri dish containing 0.22 μm -filtered water. They were then transferred into another Petri dish containing filtered water as before. This procedure rinsed away any residual host material. The parasites were then placed individually into a 1.5 ml Eppendorf tube for DNA extraction. DNA was extracted in 500 μl of 5% chelex containing 0.1 mg/ml proteinase K, incubated at 60 °C for 4 h and boiled at 100 °C for 8 min.

The mitochondrial 16S gene of all individual rediae/metacercariae sampled was sequenced and the internal transcribed spacer 1 (ITS1) nuclear gene of a subset of representative samples from each of the separate clades was also sequenced subsequently. The 16S gene was amplified using platyhelminth-specific 16S primers platy.16Sar [5'-ATCTGTTT-(A/C)T(C/T)AAAAACAT-3'] and platy.16Sbr [5'-CCAATCTTA ACTCAACTCATAT-3'] as designed by Donald *et al.* (2004). The optimum cycling parameters for these primers included an initial denaturation step of 95 °C (2 min), followed by 40 cycles of 95 °C (30 s), 48 °C (40 s) and 72 °C (1 min), followed by a final extension phase at 72 °C (10 min). The ITS1 gene was amplified using 2 primers described by Bowles and McManus (1993), BD1 [5'-GTCGTAACAAGGTTTCCGTA-3'] and 4S [5'-TCTAGATGCGTTCGAA(G/A)TGTCGATG-3']. The optimal cycling parameters for these primers included an initial denaturation of 94 °C (2 min), followed by 40 cycles of 94 °C (30 s), 52 °C (1 min) and 71 °C (1 min), followed by a final extension phase at 71 °C (10 min). The PCR products were purified using Purelink™ PCR Purification kits (Invitrogen) and sequenced using the BigDye Terminator Cycle Sequencing Kit (Applied Biosystems), and resolved with an ABI PRISM 3730 Genetic Analyser (Applied Biosystems). Sequences were aligned using ClustalW in MEGA version 3.1 genetic analysis programme (Kumar *et al.* 2004). Pairwise comparisons of sequences were conducted using Kimura's two parameter model (Kimura, 1980) also in MEGA.

Phylogenetic analyses were performed with PAUP* version 4b10 (Swofford, 2002) for maximum likelihood (ML) searches and bootstrap values (Felsenstein, 1985). To reduce computation time, for the *Acanthoparyphium* tree, only 2 sequence replicates from each haplotype were retained for the phylogenetic analyses. For the maximum likelihood analysis, the optimal model was identified using MODELTEST (Posada and Crandall, 1998), and

for bootstrap analysis, 1000 replicates with 5 random addition sequences per replicate with NNI branch swapping were used.

Bayesian analyses were performed using MrBayes v3.1.2 (Huelsenbeck and Ronquist, 2001) with the following settings: the ML employed 2 substitution types ('nst=2'). Rate variation across sites was modelled using a gamma distribution. The Markov-chain Monte-Carlo search was run with 4 chains for 500 000 generations, with trees being sampled every 100 generations (the first 1000 trees, i.e. 100 000 generations, were discarded as 'burnin').

For rooting of the *Curtuteria* phylogenetic tree, 2 representative sequences obtained for *Acanthoparyphium* were assigned as outgroup taxa. In turn, for the *Acanthoparyphium* phylogenetic tree, 2 representative sequences obtained for *Curtuteria* were assigned as outgroup taxa. The 16S and ITS1 sequence for *Fasciola hepatica* was also added as an outgroup taxon for each of the trees. *F. hepatica* was selected because of the availability of its 16S and ITS1 sequence in the GenBank database, and higher level phylogenetic analyses have found Fasciolidae to be a sister group to the Echinostomatidae (see Olson *et al.* 2003).

The Fisher's exact test was used to compare the relative incidence of the different clades among infected snails between sites located in the harbour and those from the inlets.

RESULTS

A total of 36 *Curtuteria*-infected *C. glandiformis* were collected; 1 from Deborah Bay, 17 from Lower Portobello Bay, 4 from Hooper's Inlet and 14 from Papanui Inlet (Table 1). The 16S gene of 12 *Curtuteria* metacercariae (identified based on the presence of 31 collar spines) dissected from *A. stutchburyi* collected from Lower Portobello Bay was sequenced. A total of 78 *Acanthoparyphium*-infected *Z. subcarinatus* were collected; 22 from Lower Portobello Bay, 14 from Company Bay, 14 from Turnbull Bay, and 28 from Papanui Inlet (Table 1). The 16S gene of 11 *Acanthoparyphium* metacercariae (identified based on the presence of 23 collar spines) dissected from *A. stutchburyi* and *M. liliana* collected from Lower Portobello Bay and Company Bay was sequenced. PCR amplification of 16S yielded 427 bp of readable sequence (GenBank Accession numbers FJ396045–FJ396142), for *Acanthoparyphium* and *Curtuteria*, and ITS1 yielded 555 bp of readable sequence (GenBank Accession numbers FJ396143–FJ396164) for both *Acanthoparyphium* and *Curtuteria* samples.

Parasites identified as *Curtuteria* were found to belong to 2 different clades (Table 1 and Figs 2 and 4), and *Acanthoparyphium* was found to be composed of 4 highly divergent clades (Table 1 and Figs 3 and 5). The trees produced by ML and Bayesian analyses

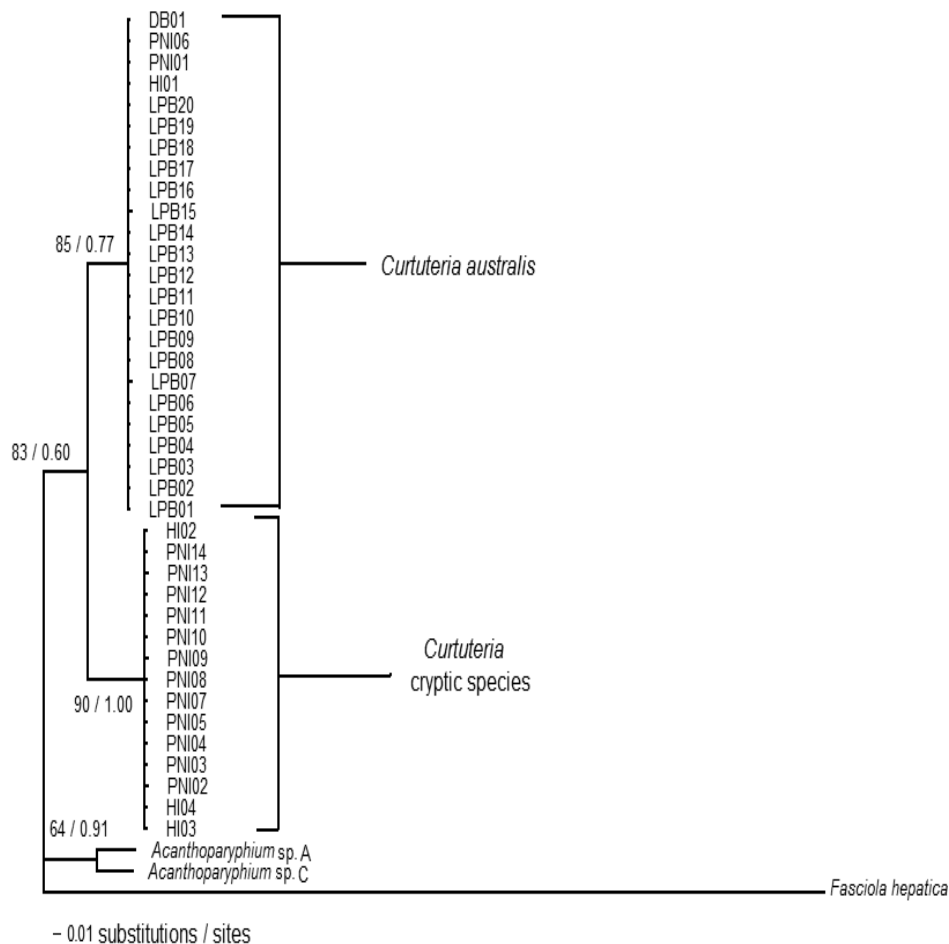


Fig. 2. Phylogenetic relationships of the *Curtuteria* spp. samples isolated from *Cominella glandiformis* inferred from 16S sequences. The codes correspond to the collection sites shown in Fig. 1. The first number associated with each node represents the ML bootstrap value, followed by the Bayesian posterior probabilities.

gave statistical support for the existence of distinct genetic species, with the topology of trees produced with data from the 16S gene (Figs 2 and 3) concordant with the trees produced using the ITS1 gene (Figs 4 and 5).

The levels of 16S sequence divergence between cryptic species were high, ranging from 13.1 to 13.7% for the 2 *Curtuteria* species (Table 2) and from 5.8 to 11.1% between the cryptic *Acanthoparyphium* species (Table 3). The slower-evolving ITS1 sequence showed considerably less divergence, with only 1.8% divergence between the 2 *Curtuteria* species (Table 2) and 0.6–1.6% divergence between the *Acanthoparyphium* species (Table 3).

The level of within clade divergence for 16S sequences was between 0.0 and 0.5% for the *Curtuteria* clades and between 0.0 and 1.1% for the *Acanthoparyphium* clades. For the ITS1 sequences, the level of divergence within each of the *Curtuteria* clades was 0.0% (Table 2), whereas within each *Acanthoparyphium* clades, the level of sequence divergence was 0.0–0.2% (Table 3).

The 16S sequences of 12 *Curtuteria* spp. metacercariae (identified based on the presence of 31

collar spines) dissected from cockles collected from Lower Portobello Bay were found to match the sequences of the 'harbour clade' of *Curtuteria* (i.e. *C. australis*) with less than 0.5% divergence. The 16S sequences of the 11 *Acanthoparyphium* metacercariae (identified based on the presence of 23 collar spines) dissected from *A. stutchburyi* and *M. liliana* collected from Company Bay and Lower Portobello Bay were found to match the sequences of *Acanthoparyphium* sp. A, with less than 0.6% divergence.

All *Curtuteria* sequenced from the harbour belong to the same monophyletic clade, while 15 out of 18 of the *Curtuteria* sequenced from the inlets were of a separate clade (Table 1 and Fig. 6A). Of the harbour sample, *Acanthoparyphium* sp. A accounted for 44 out of the 50 samples sequenced, whereas 2 were from sp. B and 4 from sp. C. In contrast, of the inlet sample, 9 out of 28 samples were of sp. A, 14 were of sp. C, and 5 were of sp. D (Table 1 and Fig. 6B). *Acanthoparyphium* sp. B was absent from the inlet sample while sp. D was absent from the harbour sample. This segregation pattern was statistically significant for both genera (Fisher's exact test, both $P < 0.0001$).

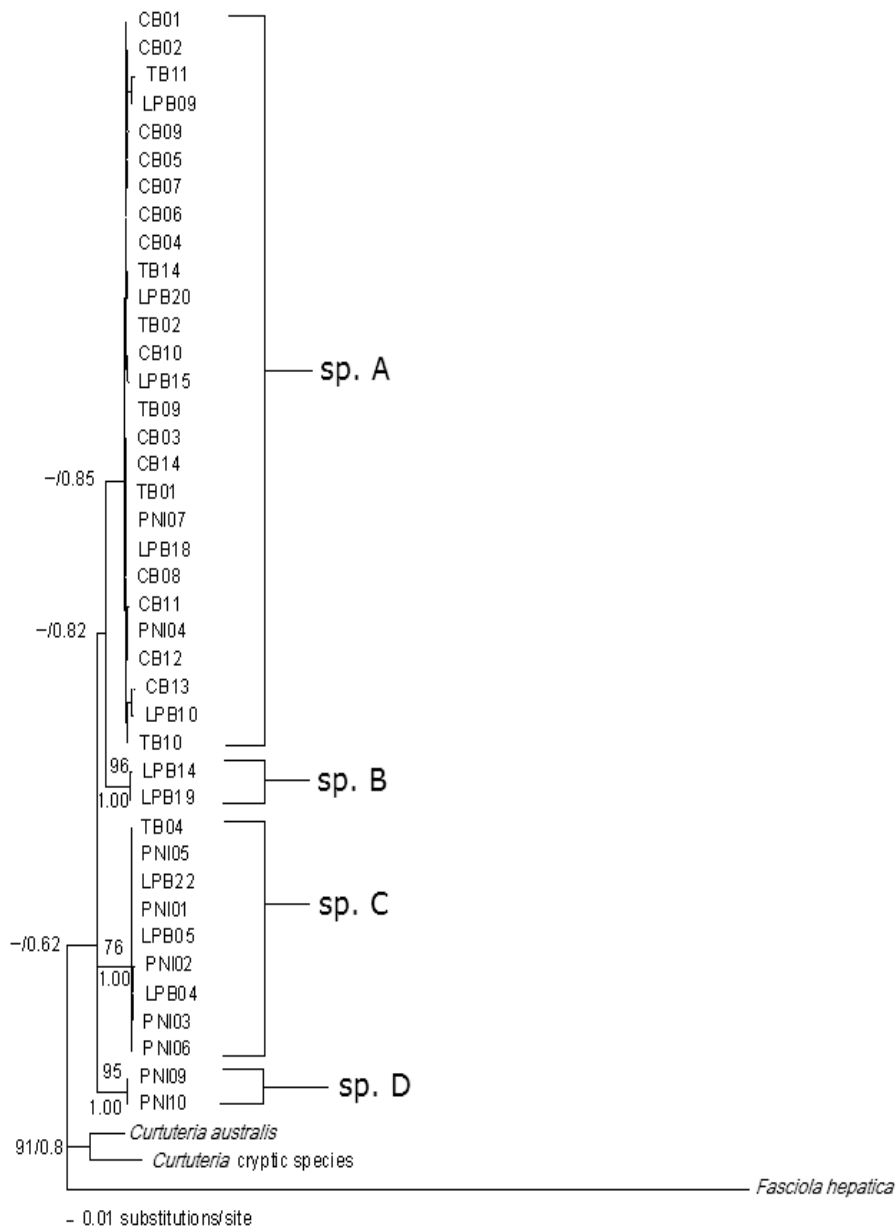


Fig. 3. Phylogenetic relationships of the *Acanthoparyphium* spp. samples isolated from *Zeacumantus subcarinatus* inferred from 16S sequences. The codes correspond to the collection sites shown in Fig. 1. The first number associated with each node represents the ML bootstrap value, followed by the Bayesian posterior probabilities.

DISCUSSION

This study has uncovered a previously unsuspected level of diversity in the trematodes of Otago Harbour in the form of genetically distinct clades within otherwise morphologically identical or very similar groups of individuals. According to Vilas *et al.* (2005), the maximum intraspecific divergence for trematodes in mitochondrial DNA sequences ranges from 0.3 to 2.2%. The levels of variation seen in this study, combined with phylogenetic support for the clades, indicate that the clades represent genetically distinct species. The level of ITS1 sequence

divergence between the different clades/species of this study are consistent with the level of interspecific variations expected for the family Echinostomatidae (reviewed by Nolan and Cribb, 2005).

The *Curtuteria* clade that encompasses all samples from whelks collected from Deborah Bay and Lower Portobello Bay, plus a few of the individuals from Hooper's Inlet and Papanui Inlet, is most likely *Curtuteria australis* described by Allison (1979). This conclusion is based on the 16S sequences of metacercariae with 31 collar spines dissected from cockles collected from Lower Portobello Bay, which closely matched those obtained from rediae dissected from

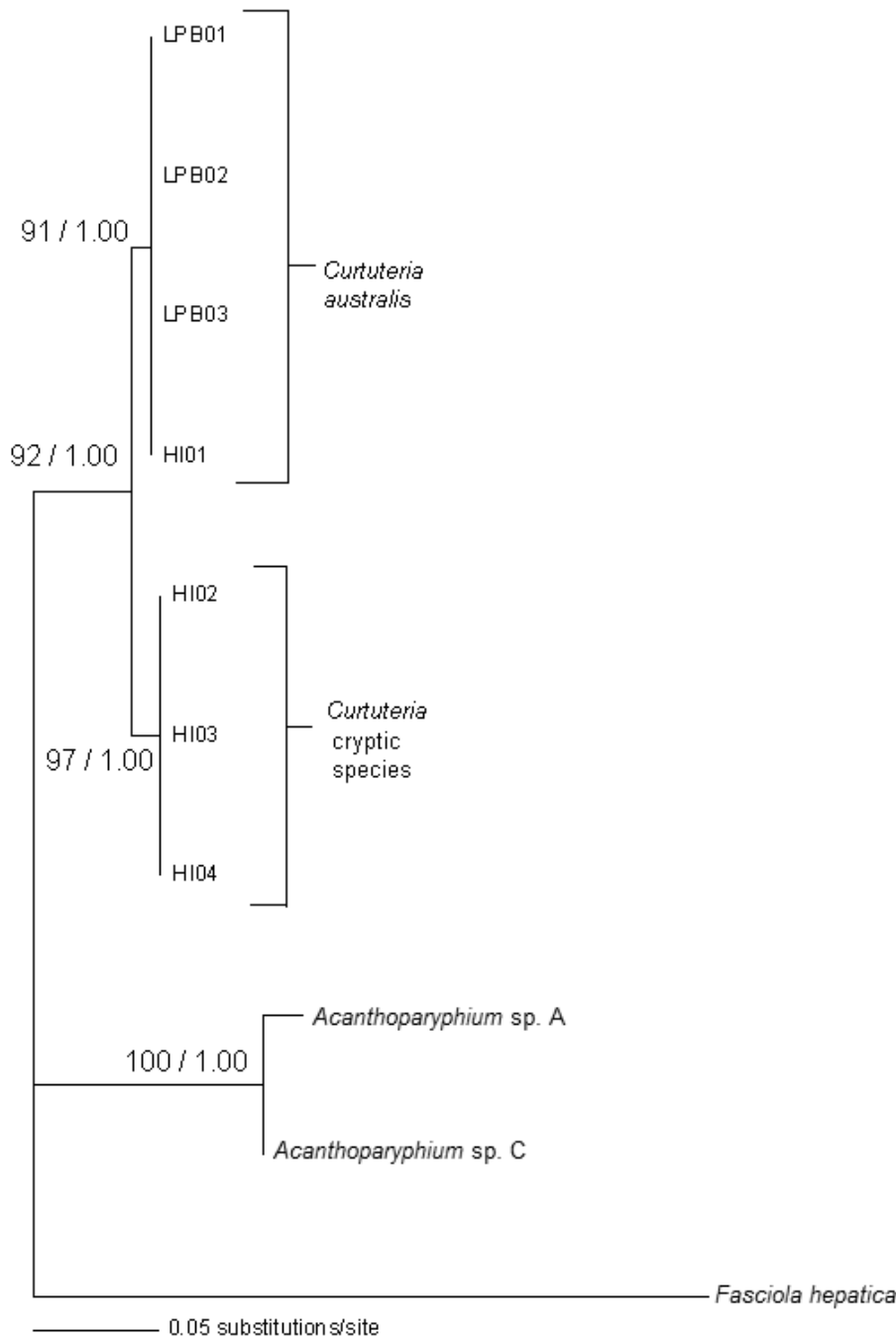


Fig. 4. Phylogenetic relationships of the *Curtuteria* spp. samples isolated from *Cominella glandiformis* inferred from ITS1 sequences. The codes correspond to the collection sites shown in Fig. 1. The first number associated with each node represents the ML bootstrap value, followed by the Bayesian posterior probabilities.

whelks and previously assigned to *C. australis* (see Babirat *et al.* 2004). The *Acanthoparyphium* metacercariae with 23 collar spines dissected from bivalves collected from Company Bay and Lower Portobello Bay were identical to sequences from *Acanthoparyphium* sp. A. This indicates that the clade that we have named sp. A in the present study most likely corresponds to *Acanthoparyphium* sp. described by Martorelli *et al.* (2006) from metacercariae.

The discovery of cryptic species of *Acanthoparyphium* adds to the growing list of trematodes known to use the mudsnail *Z. subcarinatus* as a first

intermediate host. This snail is already known to be host to at least 6 described species of trematodes (Martorelli *et al.* 2004, 2006, 2008). The results of this study not only shed new light on the diversity of trematodes infecting *Z. subcarinatus*, but also add weight to the possibility that many previously described species of trematodes found in other intertidal ecosystems in fact consist of cryptic species complexes (e.g. Miura *et al.* 2005).

The life cycles of the cryptic echinostome species found in this study are yet to be elucidated, though they are likely similar to those of *C. australis* and

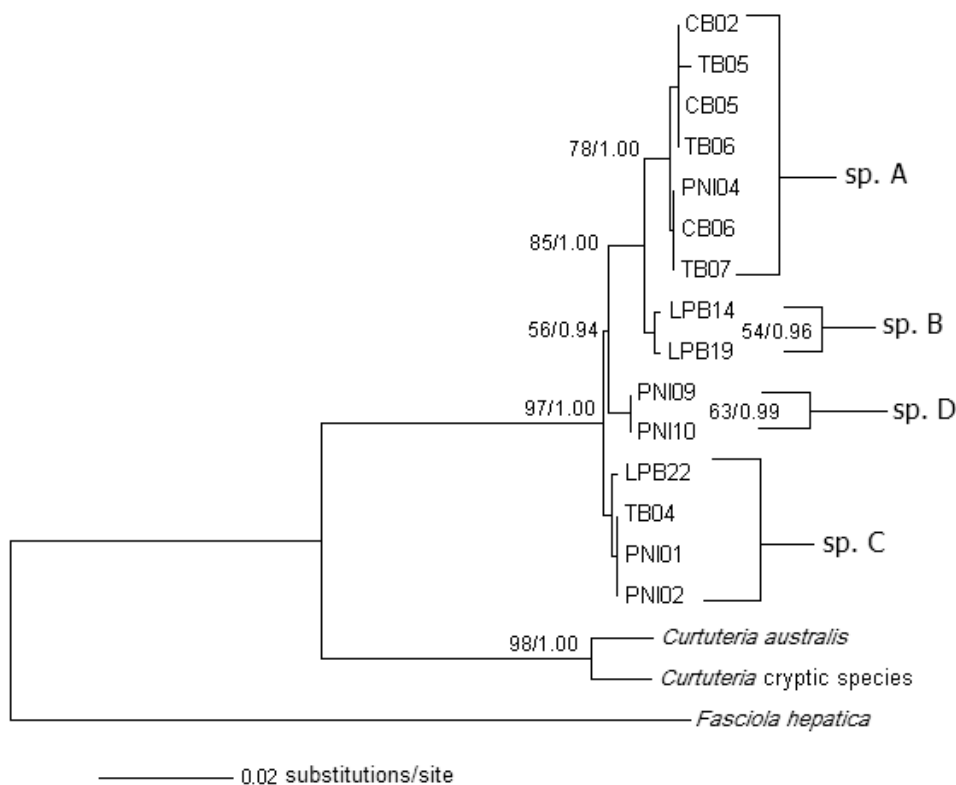


Fig. 5. Phylogenetic relationships of the *Acanthoparyphium* spp. samples isolated from *Zeacumantus subcarinatus* inferred from ITS1 sequences. The codes correspond to the collection sites shown in Fig. 1. The first number associated with each node represents the ML bootstrap value, followed by the Bayesian posterior probabilities.

Table 2. Range of percentage sequence divergence at 16S and ITS1 within and between the *Curtuteria* spp.

	<i>Curtuteria australis</i>	<i>Curtuteria</i> cryptic species
16S		
<i>Curtuteria australis</i>	0.0–0.5	13.1–13.7
<i>Curtuteria</i> cryptic species		0.0–0.5
ITS1		
<i>Curtuteria australis</i>	0.0	1.8–1.8
<i>Curtuteria</i> cryptic species		0.0

Table 3. Range of percentage sequence divergence at 16S and ITS1 within and between the *Acanthoparyphium* spp.

16S	Clade A	Clade B	Clade C	Clade D
Clade A	0.0–1.1	5.8–6.7	9.3–10.8	9.0–9.6
Clade B		0.0–0.3	10.5–11.1	9.0–9.3
Clade C			0.0–0.5	9.9–9.9
Clade D				0.0
ITS1	Clade A	Clade B	Clade C	Clade D
Clade A	0.0–0.2	0.6–0.8	1.2–1.4	1.4–1.6
Clade B		0.0	1.2–1.4	1.0–1.2
Clade C			0.2	1.1–1.2
Clade D				0.0

Acanthoparyphium sp. (sp. A). One way of elucidating their life cycles would involve prospecting for parasites from potential second intermediate hosts collected from the inlets where the cryptic clades appear to be more common. While the metacercariae of *Curtuteria* spp. are only known to infect bivalves, those of *Acanthoparyphium* can infect prosobranch and pulmonate gastropods, as well as polychaetes, in addition to bivalves (Kostadinova, 2005). The cryptic species found in this study may also infect the

cockle *A. stutchburyi*, but encyst in parts of the cockle's body other than its foot where *Acanthoparyphium* sp. A and *C. australis* co-occur. Different species of *Himasthla*, which infect the European cockle *Cerastoderma edule*, show different host tissue preferences for encystment (Wegeberg *et al.* 1999; de Montaudouin *et al.* 2005); therefore, the presence of the cryptic species in *A. stutchburyi* may simply have been overlooked. However, all echinostome metacercariae found in cockles in the Otago area show a specific preference for the tip of the host's foot (Mouritsen 2002; Babirat *et al.* 2004) and rarely

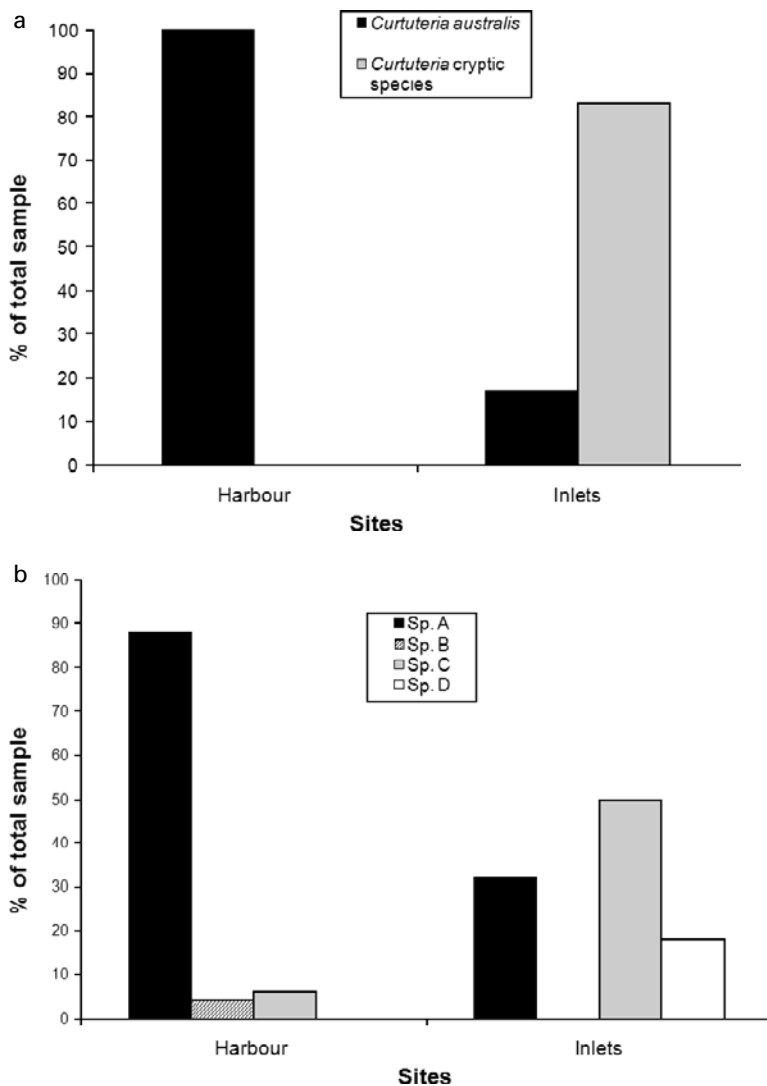


Fig. 6. The relative incidence of the *Curtuteria* spp. (A) and the *Acanthoparyphium* spp. (B) among infected snails with respect to their site of origin.

encyst elsewhere. Site segregation within the cockle host between these cryptic species may occur at a much finer scale, i.e. within different subsections of the foot. Mouritsen (2002) found that the foot-encysting echinostomes in cockles alter the host's burrowing behaviour by simple mechanical obstruction, and although metacercariae occur throughout the cockle's foot, only those encysted at the tip of the foot actually impair host burrowing. In addition, the manipulation of cockle behaviour comes at considerable risk in the form of predation by non-host fish predators that crop the tip of the cockle's foot (Mouritsen and Poulin, 2003). While previous findings suggest that both *Acanthoparyphium* sp. and *C. australis* contribute equally to host manipulation by preferentially encysting at the tip of the cockle's foot (Babirat *et al.* 2004), in light of the present results, it is possible that niche partitioning is occurring between the cryptic species of echinostomes. Certain cryptic species may be 'hitch-hikers' that do

not manipulate host behaviour, but associate themselves with parasites that do alter host phenotype (Thomas *et al.* 1998b). By encysting away from the tip, these metacercariae can exploit the enhanced transmission rate induced by manipulative clades, but without incurring the fish predation risk associated with host manipulation.

Apart from the discovery of 6 cryptic species among trematodes previously described as just 2 species, our most striking finding is the extremely localized pattern of distribution of these species. For example, while the cryptic *Curtuteria* species is 5 times more common than *C. australis* in sheltered inlets, it is either absent or very rare in the Otago Harbour. A similar small-scale spatial segregation pattern is also apparent among the *Acanthoparyphium* species. This is particularly perplexing considering that the definitive hosts of these trematodes are birds (Kostadinova, 2005) that can disperse infective stages across wide geographical areas

(Criscione and Blouin, 2004; Miura *et al.* 2006; Keeney *et al.* 2008). The marked segregation of these cryptic species on a scale of a few kilometres therefore warrants a discussion of potential explanations.

First, and most parsimoniously, the patterns seen in Fig. 6 could simply be the result of a sampling artefact, especially given the small sample size for the *Curtuteria* species. However, this pattern is repeated in *Acanthoparyphium* spp. for which a larger number of individuals were sequenced. While a larger sample would further clarify the distribution pattern of these cryptic species, regardless of sample size, the fact that 2 different genera that utilize 2 different species of gastropod as first intermediate hosts display a similarly uneven distribution makes it difficult to dismiss as a mere by-product of inadequate sampling.

Secondly, the distribution pattern may be the consequence of local adaptation: either by the parasite towards a particular host population, or by a host population against a particular parasite. While trematodes are highly specific for their first intermediate host (Gibson and Bray, 1994), mainly because of molluscan defence responses (Sapp and Loker, 2000; Bayne *et al.* 2001), this specificity does not extend to specialization on a particular host population. Local adaptation on the part of the parasite seems even less likely given that avian definitive hosts can disperse the larval stages over great distances, resulting in genetically homogeneous parasite populations (Criscione and Blouin, 2004; Keeney *et al.* 2008). Local adaptation by the gastropod host appears more likely. Because both *Z. subcarinatus* and *C. glandiformis* produce crawling larvae without planktonic veliger stages (Pilkington, 1974), gastropods within either the bays or inlets could represent isolated populations that vary in their susceptibility to infection by different trematode species. Lively and Jokela (1996) have reported that freshwater snails of the same species sampled along a depth gradient in a single lake varied in their susceptibility to infection by the same trematode species. However, while populations of *Z. subcarinatus* from bays with high parasite prevalence exhibit life-history adaptations that minimize the negative impact of trematode infection, such as maturing at a smaller size, no variation was found in the susceptibility of snails from these different populations to trematode infections (Fredensborg and Poulin, 2006).

Thirdly, differences in environmental conditions between the harbour and inlets could give certain species a competitive advantage over others in terms of successfully infecting the appropriate molluscan host. Several habitat features can contribute to spatial heterogeneity in species composition of snail-trematode communities (Williams and Esch, 1991; Koprivnikar *et al.* 2007). Both biotic and abiotic factors affect the survival of free-living stages of endohelminths, including trematodes (Pietroock and

Marcogliese, 2003; Thieltges *et al.* 2008). However, these environmental conditions generally have broad-spectrum effects with very little specificity, certainly not enough to target one species of a genus but not another. It is difficult to pinpoint potential environmental factors that differ between the harbour and inlets and that could only affect a particular species while not affecting congeners, let alone having a similar impact on another genus. Thus, differences in local factors, both abiotic and biotic, are unsatisfactory as potential explanations for the observed spatial segregation of cryptic species.

Fourthly, while the environment may not be directly responsible for the segregated distribution of these species, because they use the definitive host as their main means of dispersal, environmental factors may still shape distribution patterns by influencing the spatial distribution of shorebirds. There is a positive association between habitat usage by shorebirds and the recruitment rate of trematodes in local snail populations (Smith, 2001; Hechinger and Lafferty, 2005; Fredensborg *et al.* 2006; Whitney *et al.* 2007; Byers *et al.* 2008). Thus the presence of birds at a particular site could modulate the subsequent distribution of different trematode species. Habitat choices of shorebirds when selecting a site for feeding, breeding, or roosting are influenced by a number of factors including substrate characteristics (Mouritsen and Jensen, 1992; Finn *et al.* 2007) and prey availability (Ribeiro *et al.* 2004; Jing *et al.* 2007), which in turn can be affected by the presence of bioturbators such as burrowing crustaceans (Iribarne *et al.* 2005). Divergent site preferences by different shorebird species or individuals infected by different parasite species could possibly maintain the localized distribution seen in the two echinostome species complexes.

None of the above is a very likely or convincing explanation, however. The limited distance between Otago Harbour and the surrounding inlets make it difficult to attribute the observed distribution pattern to bird movement. The somewhat unexpected patterns found here present a tantalizing glimpse into a biological puzzle that warrant further and more intensive investigation. Future approaches should include: larger sample sizes, elucidation of the life cycles of all cryptic species, development of a faster and cheaper method of identifying the cryptic species (such as RFLP; Donald *et al.* 2007) since cockles commonly harbour hundreds of echinostome metacercariae (Leung and Poulin, 2007), and possibly conducting bird surveys to ascertain if host movements can generate the distribution pattern found for the cryptic species. Assuming that the present findings are valid, we may have to reconsider some of the accepted but largely untested tenets regarding the trematode life cycle with respect to host specificity and dispersal potential.

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ERRATUM

Cryptic species complexes in manipulative echinostomatid trematodes: when two become six – ERRATUM

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A new Table 1 has been supplied by the authors in which the number of samples from Papanui Inlet and the Total number of samples in the last two lines of the table have been corrected.

Table 1. Sampling site and number of *Curtuteria* spp. and *Acanthoparyphium* spp. samples

Sampling site	<i>Curtuteria</i>		<i>Acanthoparyphium</i>			
	<i>Curtuteria australis</i>	<i>Curtuteria</i> cryptic species	Sp. A	Sp. B	Sp. C	Sp. D
Company Bay	0	0	14	0	0	0
Deborah Bay	1	0	0	0	0	0
Lower Portobello Bay	17	0	17	2	3	0
Turnbull	0	0	13	0	1	0
Hooper's Inlet	1	3	0	0	0	0
Papanui Inlet	2	12	9	0	14	5
Total	21	15	53	2	18	5