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Distributions of two ectosymbionts, branchiobdellidans (Annelida: Clitellata) and scutariellids (Platyhelminthes: “Turbellaria”: Temnocephalida), on atyid shrimp (Arthropoda: Crustacea) in southeast China

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Distribution of two ecologically similar but usually spatially separate ectosymbionts, branchiobdellidans (Annelida) and scutariellids (Platyhelminthes), on atyid shrimp (*Neocaridina* spp.) is reported from 18 localities in five Provinces of southeastern China. Prevalence was determined for the branchiobdellidan, *Holtodrilus truncatus*, found at seven locations, the scutariellid, *Scutariella japonica*, present at every site, and where cohabitation occurred. Both ectosymbionts showed a microhabitat predilection for the host's branchial chambers and instances of cohabitation occurred at all seven locations where *H. truncatus* were collected, although not on every shrimp. On-site observations of live hosts supporting both ectosymbionts showed that neither *H. truncatus* nor *S. japonica* reacted aggressively or defensively towards the other when in close proximity. Instances of imported Chinese *Neocaridina* spp. into central Honshu Island, Japan, almost certainly came from areas in southeast China identified in this study. These imported populations are predicted to spread northwards into the area where endemic Japanese branchiobdellidans occur.

Keywords: Branchiobdellida; Temnocephalida; shrimp; cohabitation; China

Introduction

Two non-pathogenic, ectosymbiont taxa, Branchiobdellida (Annelida: Clitellata) and Temnocephalida (Platyhelminthes: “Turbellaria”) independently evolved a similar ectosymbiotic life style on their respective crustacean hosts, with endemic branchiobdellidans having a disjunct Holarctic, and temnocephalidans a largely disjunct Gondwanan distribution (Gelder 1999). Members of the Branchiobdellida on astacoidean crayfish have been reported in North and Central America (Gelder et al. 2002; Govedich et al. 2010), East Asia (Timm 1991; Gelder and Ohtaka 2002), and the Euro-Mediterranean region (Gelder 2006; Fard and Gelder 2011). However, branchiobdellidans have extended beyond the southerly endemic range of crayfish into

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Central America and south and central China, where they have adopted freshwater crabs, shrimps and other crustaceans as hosts (Gelder 1999; Gelder et al. 2002). The order Temnocephalida is divided into two families: Temnocephalidae is found in South and Central America, Madagascar and Australasia, while members of the Scutariellidae are absent from the Americas, but distributed along the southern margin of the Alpine–Himalayan mountain range from Italy through the Balkans, Ukraine, India then north across southern China and Japan (Gelder 1999; Cannon and Joffe 2000). In contrast to branchiobdellidans, scutariellids are primarily found on shrimp hosts.

East Asia presents a unique model for studying ectosymbiont range extensions that overlap, and in particular branchiobdellidans from the north and scutariellids from the south (Gelder 1999). Branchiobdellidans on crayfish hosts have been reported across northern China, the Korean Peninsula, southeast Russia and northern Japan (Gelder and Ohtaka 2002) with two monotypic genera occurring on atyid shrimps in central and southern China. These monotypics are *Caridinophilus unidens* Liang, 1963 on *Caridina yunnanensis* Yu, 1938, in Yunnan Province and *Holtodrilus truncatus* (Liang, 1963) on *Neocaridina denticulata sinensis* (Kemp, 1918), in Henan (Liang 1963) and Guangdong (Liu 1984) Provinces. Atyid and palaemonid shrimps are common across central and southern China and Japan, and Korea. Some shrimps have been reported to carry *Scutariella japonica* (Matjašič, 1990); specifically *Caridina* sp. in the Lijiang River, Guangxi Zhuang Autonomous Region (Matjašič 1990) and Lake Taihu, Jiangsu Province (Kemp 1918), China, and on *Paratya compressa* (De Haan, 1844) in and near Lake Biwa, Japan (Kobayashi 1935; Honjō 1937). Recently Ohtaka and Chen (2010) had difficulty separating populations of *N. d. sinensis* from *N. d. denticulata* (De Haan, 1844) in collections from China, Korea, Taiwan and Japan using morphological characters and molecular sequences of cytochrome oxidase I (COI) and 16S ribosomal RNA (Ikeda et al., unpublished data). As these shrimp may form a species complex, this study refers to them as *Neocaridina* spp. to prevent further taxonomic confusion.

Although branchiobdellidan and scutariellid ranges overlap in central and southern China, this is not true in Japan where branchiobdellidans occur on Hokkaido and northern Honshu Islands, and are separated from scutariellids in central Honshu by 500 km. This endemic distribution was complicated when Niwa et al. (2005) reported the first occurrence of a mainland branchiobdellidan, *H. truncatus*, in central Honshu Island on *Neocaridina* spp. suspected of being imported from China. The situation was further complicated when imported live *Neocaridina* spp. carrying scutariellids were observed arriving at Kansai Airport in Kinki District, Japan, from an unspecified location in China (Niwa and Ohtaka 2006). Additional collections made on central Honshu Island revealed that introduced *Neocaridina* spp. populations were carrying both *H. truncatus* and *S. japonica* at 10 sites in the Kinki District (Niwa and Ohtaka 2006). Searches were then made for *Neocaridina* spp. on other offshore islands and resulted in *H. truncatus* being collected from Taiwan (Ohtaka and Chen 2010) and Miyako Island, Japan (Fujita et al. 2010); whether these populations are endemic or introduced remains to be determined.

The primary aim of this study was to establish the distribution of branchiobdellidan and scutariellid species in southeastern China as the most likely area of ectosymbiont distribution overlap and source of shrimp introduced into Japan. Collecting methods were chosen that would also provide information on prevalence, microhabitat preferences and behaviour of the ectosymbionts. This information would

then be available for comparison in future studies on these taxa, which have been commercially introduced into Japan and other countries.

Materials and methods

Neocaridina spp. shrimp were collected from 18 lotic and lentic sites in five Provinces of southeastern China during July or August over three years: 2007, 2008 and 2009 (Table 1). Dip-nets (mesh size 0.5 mm) were dragged through aquatic vegetation with displaced shrimp being captured in the nets. Between 34 and 109 live shrimp were randomly selected from the catch taken at a locality and each specimen was placed individually into a 2.0-ml plastic tube filled with 5% formalin preserving solution.

Each shrimp was examined in a laboratory under a dissection microscope, and numbers and locations of branchiobdellidans and scutariellids on hosts or in bottom debris were recorded. The locations of the two ectosymbionts' cocoons were also recorded. Selected mature specimens of both worms were washed in water, dehydrated in a graded ethanol series, cleared in methyl salicylate and mounted in Canada balsam on a slide (Govedich et al. 2010). Branchiobdellidans were identified using Liang (1963) and scutariellids, Matjašič (1990).

Behaviour of both ectosymbionts, separately and together, was observed in a shrimp's branchial chamber while in a Petri dish under a dissection microscope. Ten shrimp from the Nanshui River, Guangdong Province (site 4), were monitored separately with each viewing lasting 10 to 30 minutes.

Results

A total of 1681 *Neocaridina* spp. were collected from 18 freshwater sites in southeast China. Most of these shrimp hosted one or two ectosymbiotic worms; a branchiobdellidan, *Holtodrilus truncatus* (Liang, 1963) (Figure 1A) and a scutariellid, *Scutariella japonica* (Matjašič, 1990) (Figure 1B), revealing that they were more widely distributed than previously reported. Although *S. japonica* was found at all 18 sites, *H. truncatus* occurred only at seven localities: Guangdong (sites 2–4), Anhui (site 13) and Zhejiang (sites 15, 16, 18) Provinces (Table 1, Figure 2).

Prevalence of *S. japonica* ranged from 25.2% in Nanshui River (site 4) to 100% at Fudi (site 10), whereas that of *H. truncatus* was much lower, 1.0–37.0%. Cohabitation was found at all seven locations supporting branchiobdellidans, with a prevalence range of 1.0 to 27.0%, occurring on 64% of the shrimps carrying *H. truncatus*, and 19% of those with *S. japonica*. These data are presented in full in Table 1 and Figure 2.

Both *H. truncatus* and *S. japonica* showed a microhabitat predilection for the host's branchial chambers, whether separately or cohabiting, with occasional individuals being found on the host's exposed body surface. Supporting evidence for such a preference was provided when 71 shrimp carrying identifiable cocoons were examined. Those cocoons from *H. truncatus* were attached to the host by a peduncle or stalk, whereas those from *S. japonicus* lacked a peduncle. All branchiobdellidan cocoons were found on the gills, and although this was largely true for scutariellid cocoons, others were scattered over the host's exposed surface. Particular attention was paid to the cuticle surface over vulnerable areas such as gills and egg cases of brooding females during exoskeleton examinations and they were found to be smooth and healthy.

Table 1. Location of collection sites and dates in SE China with prevalence of *Neocaridina* spp. hosts carrying *Holtodrilus truncatus*, *Scutariella japonica* and both ectosymbionts expressed as percentages.

| Site No. | Locality | Location | | Date | No of <i>Neocaridina</i> spp. examined | Prevalence (%) | | |
|------------------------------|-------------------------------------|--------------|---------------|--------------|--|---------------------|--------------------|-----------|
| | | Latitude (N) | Longitude (E) | | | <i>H. truncatus</i> | <i>S. japonica</i> | Both taxa |
| Shaoguan, Guangdong Province | | | | | | | | |
| 1 | Stream 1 in Longnan Town | 24°43'26.4" | 113°09'39.5" | 30 Aug. 2007 | 101 | 0 | 92.1 | 0 |
| 2 | Stream in Ruyuan county | 24°46'14.7" | 113°15'26.4" | 30 Aug. 2007 | 109 | 16.5 | 86.2 | 15.6 |
| 3 | Stream 2 in Longnan Town | 24°44'23.7" | 113°10'52.1" | 31 Aug. 2007 | 94 | 22.3 | 52.1 | 17.0 |
| 4 | Nanshui River in Longgui Town | 24°44'30.5" | 113°23'33.4" | 31 Aug. 2007 | 103 | 29.1 | 25.2 | 7.7 |
| Fujian Province | | | | | | | | |
| 5 | Bantou Reservoir in Houxi Town | 24°41'54.0" | 117°59'21.5" | 7 July 2008 | 34 | 0 | 50.0 | 0 |
| 6 | Stream in Putian City | 25°27'44.3" | 119°00'21.9" | 7 July 2008 | 92 | 0 | 62.0 | 0 |
| 7 | Stream in Longhua Town | 25°19'01.8" | 118°35'47.1" | 9 July 2008 | 74 | 0 | 33.8 | 0 |
| 8 | Stream in Laoying, Liancheng County | 25°48'00.8" | 116°44'50.3" | 13 July 2008 | 96 | 0 | 94.8 | 0 |
| 9 | Stream in Qiulai, Liancheng County | 25°46'21.2" | 116°41'52.2" | 13 July 2008 | 90 | 0 | 82.2 | 0 |

| | | | | | | | | | |
|-------------------|----------------------------------|-------------|--------------|--------------|-----|------|------|------|--|
| 10 | Stream in Fudi, Shanghang County | 25°40'47.3" | 116°46'12.1" | 13 July 2008 | 84 | 0 | 100 | 0 | |
| Jiangxi Province | | | | | | | | | |
| 11 | Pond in Jiujiang City | 29°40'37.6" | 116°03'21.8" | 3 July 2009 | 100 | 0 | 36.0 | 0 | |
| 12 | Lake Poyang in Xingzi County | 29°27'54.2" | 116°01'30.8" | 4 July 2009 | 100 | 0 | 78.0 | 0 | |
| Anhui Province | | | | | | | | | |
| 13 | Stream in Lantian Town | 29°55'39.6" | 118°05'25.5" | 7 July 2009 | 100 | 1.0 | 65.0 | 1.0 | |
| 14 | River in Xiuning County | 29°47'30.4" | 118°09'55.5" | 8 July 2009 | 100 | 0 | 80.0 | 0 | |
| Zhejiang Province | | | | | | | | | |
| 15 | Stream in Chang Hua Zhen | 30°10'45.6" | 119°13'13.4" | 8 July 2009 | 100 | 37.0 | 58.0 | 27.0 | |
| 16 | Stream in Kuntong Village | 30°45'30.2" | 119°47'19.7" | 9 July 2009 | 100 | 1.0 | 85.0 | 1.0 | |
| 17 | Stream in Hengcun Town | 29°50'55.4" | 119°33'20.6" | 10 July 2009 | 100 | 0 | 57.0 | 0 | |
| 18 | Stream in Huabu Town | 29°00'06.7" | 118°17'04.7" | 10 July 2009 | 104 | 26.0 | 70.2 | 16.3 | |

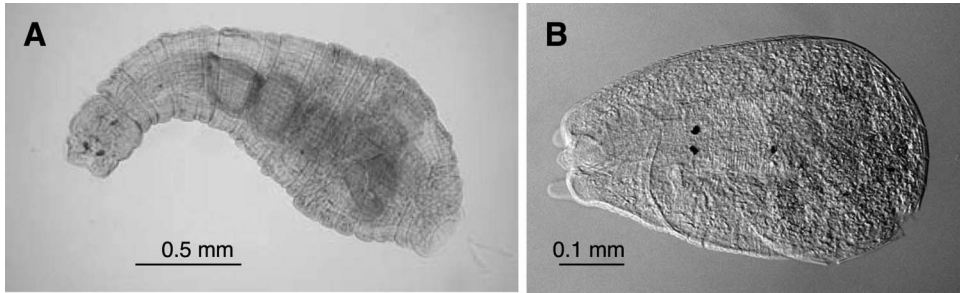


Figure 1. Photomicrographs of slide mounted ectosymbionts from *Neocaridina* spp. shrimp in southeast China: (A) *Holtodrilus truncatus* (Branchiobdellida); (B) *Scutariella japonica* (Temnocephalida).

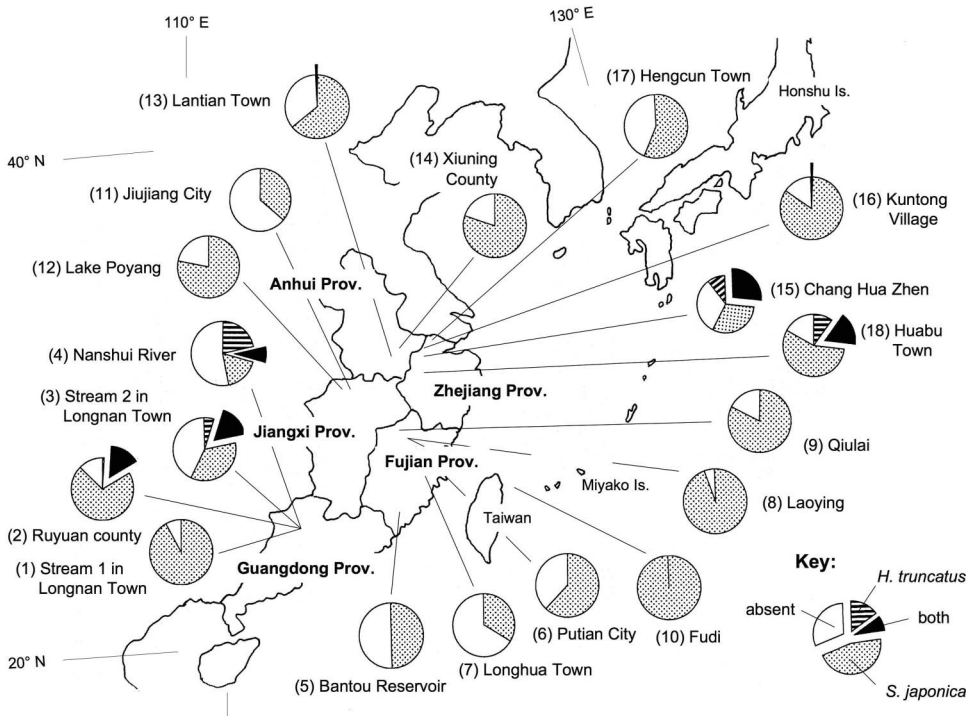


Figure 2. Distribution of *Neocaridina* spp. collection sites (numbered) in southeast China with pie charts depicting prevalence of branchiobdellidans (horizontal lines), scutariellids (dotted), both (solid), and absent (open); data from Table 1.

When not moving around, *H. truncatus* attached their posterior sucker inside the branchial chamber and allowed the anterior body to extend beyond the carapace and wave posteriorly. By contrast, *S. japonica* was quite mobile but usually stayed covered within the chamber. There was little free space in the branchial chamber, so when both ectosymbionts cohabited, they regularly came into close contact with each other. About 30 such encounters were observed, during which neither of the two ectosymbionts made contact nor showed any aggressive behaviour towards the other.

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Discussion

Previously *H. truncatus* and *S. japonica* were reported in Henan (Liang 1963), and neighbouring Jiangsu Provinces (Kemp 1918), respectively, in east central China, whereas in the south the branchiobdellidan was in Guangdong Province (Liu 1984) and the scutariellid was in adjacent Guangxi Province (Matjašič 1990). Although reasonable to assume these ectosymbionts would be present in the intervening provinces, this study was needed to provide confirmation. Results showed that scutariellids were common across the region with branchiobdellidans being more localized. Variations in ectosymbiont prevalence at a site and between sites suggest that each occurrence is the result of a number of factors, probably both behavioural and ecological, which will require detailed study to elucidate. Some of these behavioural factors may also be involved in the unique cohabitation of *H. truncatus* and *S. japonica* on *Neocaridina* spp.

Both ectosymbionts appear to have a microhabitat preference for gill chambers, although this is not exclusive because a few individuals occurred on the host's body surface. An absence of damage to host gills and egg cases supports the idea that the associations are not parasitic, contrary to inferences based on branchiobdellidans that are gill-dwelling (Holt 1973). However, nutritional relationships between host and ectosymbionts remain speculative.

This is the first study to report on-site observations on interactions of live *H. truncatus* and *S. japonica* in the host's branchial chamber, and where cohabitation occurred and appeared to be completely harmonious. This relationship was contrary to laboratory observations made when four Italian scutariellids, *Bubalocerus pretneri* Matjašič 1958, removed from cave-dwelling *Troglocaris* sp. shrimp, were artificially placed one at a time on the surficial carapace of the crayfish *Austropotamobius pallipes* (Lereboullet, 1858), carrying a *Branchiobdella astaci* Odier, 1823. Two *Bubalocerus pretneri* were rapidly ingested by the branchiobdellidan, one was partially ingested but escaped, and a fourth escaped without making contact (Gelder 1999). Although these scutariellids readily attached to the crayfish, *Branchiobdella astaci* actively refused to become attached to a shrimp despite repeatedly being placed on its carapace. Whereas spatial separation of branchiobdellidan–crayfish and scutariellid–shrimp associations exists in Italy and adjacent countries, both associations are present in surface waters in Montenegro and Macedonia to the south (Gelder 1999). Under these circumstances, experimental observations would suggest that any successful adoptive moves by scutariellids onto crayfish would be short-lived because of branchiobdellidan predation. However, whatever phenomena caused *Branchiobdella astaci* to exhibit predatory behaviour and reject *Troglocaris* sp. in Italy do not operate in the *H. truncatus* and *Caridinophilus unidens* shrimp associations in China (Liang 1963). This tolerance for non-crayfish hosts is also seen in a few North American branchiobdellidans that have adopted freshwater isopods, shrimps and crabs (Gelder et al. 2002; Gelder and Messick 2006).

The current preliminary study has obtained new information that has helped to answer some existing questions and provided a basis for future investigations into distributions and ecology of branchiobdellidan–scutariellid–shrimp associations while tracing exotic species introductions and their impact on endemic associations. Although the distribution of *H. truncatus* and *S. japonica* on *Neocaridina* spp. in southeastern China has become clearer, their limits within China remain to be determined by future sampling. However, the widespread distribution of *S. japonica* on *Neocaridina* spp. in southeastern China established by this study virtually guarantees

that these ectosymbionts would be introduced into Japan, or any other region, when the shrimp are exported. Suspicions already existed when Niwa and Ohtaka (2006) observed Chinese *Neocaridina* spp. carrying *S. japonica* being unloaded at Kansai Airport, Japan, although their collection site was not known. Subsequent studies have shown that scutariellid–shrimp association is now widespread on Honshu, Kyushu and Okinawa Islands (Kawakatsu et al. 2007–2011; Nishino et al. unpublished data), and further introductions are assured given the popularity of sport fishing and the use of *Neocaridina* spp. as bait (Niwa and Ohtaka 2006). The discovery of mainland *H. truncatus* on *Neocaridina* spp. (Niwa et al. 2005) followed by finding both *H. truncatus* and *S. japonica* cohabiting at 10 sites in the Kinki District, central Honshu Island, Japan (Niwa and Ohtaka 2006), confirmed that they had been imported, most likely from one of the two regions in southeast China shown in Figure 2. A difficult challenge will be determining which scutariellid–shrimp associations in Japan are endemic, and which result from commercially imported Chinese *Neocaridina* spp. (Niwa and Ohtaka 2006) that have escaped into the wild. This will require a carefully planned monitoring programme and the development of a molecular sequencing protocol capable of differentiating between Japanese and Chinese specimens. Such a protocol may also help to determine whether the *H. truncatus*–shrimp association found in Taiwan and Miyako Islands, Japan, is endemic or exotic from southeastern China.

Given the prediction that the Chinese ectosymbiont–shrimp association will continue to spread, it is only a matter of time before the association extends into northern Honshu and Hokkaido Islands where Japan's endemic branchiobdellidans occur. Information from the current study on ectosymbiont prevalence, apparent microhabitat preference and interactional behaviour where cohabitation occurred, will be important in estimating and then evaluating the effects on the endemic symbioses. Therefore further studies on Chinese ectosymbiont–shrimp associations will collect biotic and abiotic data to determine what effect they have on ectosymbiont population structure. Only with these data can a realistic conservation strategy be developed to minimize the impact of imported Chinese shrimp associations on endemic Japanese freshwater species.

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