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ORIGINAL PAPER

Surface glycoproteins are not the contact pheromones in the *Lysmata* shrimp

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Abstract Behavioral evidence suggests that some male caridean shrimp, such as those of *Lysmata* species, identify conspecific females via contact pheromones that coat the cuticle surface of the females. In this study, we attempted to determine whether the contact pheromones in three *Lysmata* species, *Lysmata ankeri*, *Lysmata boggessi*, and *Lysmata wurdemanni*, are glycoproteins as hypothesized previously in a diverse group of aquatic invertebrates. Twenty lectins were screened and lectin-binding experiments indicated that lectin treatment did not affect mate recognition in the shrimps. The behavior of the male-phase (MP) shrimp in the three treatments (non-lectin-treated MP and lectin-treated euhermaphrodite-phase (EP) shrimp, lectin-treated MP and lectin-treated EP shrimp, and lectin-treated MP and non-lectin-treated EP shrimp) and in the control was not different in responding to lectin-treated and control EP shrimp. All the MP shrimp copulated with lectin-treated and control EP shrimp successfully. All the MP shrimp copulated with ethylenediamine tetraacetate-treated EP shrimp (with glycoproteins removed from their cuticle surface) immediately after they detected the EP shrimp. The results suggest that glycoproteins are not likely to be the contact sex pheromones in the three *Lysmata* shrimp species.

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Introduction

The key role of sex pheromones in mate recognition of many decapod crustaceans has been well documented (Dunham 1978, 1988 for reviews). In many groups, such as crabs (Ryan 1966; Gleeson 1980; Seifert 1982; Hardege et al. 2002), lobsters (Atema 1984 for a review), and crayfish (Ameyaw-Akumfi and Hazlett 1975; Tierney et al. 1984), the urine of females contains a soluble pheromone that acts over a distance to attract mating partners. However, as Burkenroad (1947) proposed, some shrimp may, in addition to distance cues, possess contact pheromones (substance coating female's cuticle), allowing a male to recognize the receptive female (Kamiguchi 1972; Bauer 1979). Behavioral evidence suggests the existence of such contact pheromones in the caridean shrimps *Palaemonetes pugio* (Caskey and Bauer 2005), *Lysmata ankeri* (personal observation), *Lysmata boggessi* and *Lysmata wurdemanni* (Zhang and Lin 2006).

Shrimps in the genus *Lysmata* have attracted much attention because they have an unusual reproductive system, protandric simultaneous hermaphroditism (see Bauer 2000 for a review). To date, all these studies indicate that individuals in the genus first develop into a male-phase and then may change sex to a euhermaphrodite-phase (termed

female-phase by Bauer and his colleagues, or simultaneous hermaphrodite by R. Calado) with both male and female functions (Bauer 2000 for a review). The intermolt euhermaphrodite-phase shrimp that functions as a male is able to mate with the newly molted euhermaphrodite-phase shrimp that plays the female role. Male-role shrimp (MP or intermolt EP) of *L. ankeri*, *L. boggessi*, and *L. wurdemanni* have an active pre-copulatory behavior that indicates these shrimp track and locate the receptive females by both distance and contact pheromones (Zhang and Lin 2006; personal observation).

Contact pheromones may play a series of important ecological roles, such as in mate recognition, kin recognition, sexual selection, and preventing gene exchange between sibling taxa (e.g. Higgie et al. 2000; Shine et al. 2002; Howard et al. 2003). Contact sex pheromones are well known and have been identified in a variety of insect species including *Drosophila* where they were identified as cuticular hydrocarbons (e.g. Linn and Roelofs 1995; Etges and Jackson 2001; Ginzel et al. 2003; Zhang et al. 2003; Sugeno et al. 2006), but they have not been well investigated in aquatic invertebrates, including crustaceans (Fletcher and Hardege 2009). In aquatic animals, it has been found that a diverse group of invertebrates use surface glycoproteins as contact chemical recognition signal. For example, sea urchin and bivalve gamete recognition depends on glycoproteins (Glabe and Clark 1991; Lopez et al. 1993; Focarelli and Rosati 1995). A marine rotifer (Snell et al. 1995) and some copepods (Snell and Carmona 1994; Kelly and Snell 1998; Ting et al. 2000) use glycoproteins as mate recognition signal. Copepods are the only group of crustaceans reported to use glycoproteins as contact cue for mate recognition (Snell and Carmona 1994; Kelly and Snell 1998; Ting et al. 2000). Results from the studies on copepods indicate that lectins bind to surface glycoproteins on females and obscure male mate-recognition (Lonsdale et al. 1996) and treating adult males of the marine harpacticoid *Tigriopus japonicus* with the *Triticum vulgaris* lectin significantly inhibited mate recognition and guarding behavior (Kelly and Snell 1998). Glycoproteins also exist on the cuticular surface of decapod crustaceans (Marlowe et al. 1994; Shafer et al. 1994), but whether decapod crustaceans also use cuticular glycoproteins as contact cues in mate recognition is unknown.

In this study, we tested whether three *Lysmata* species use cuticular glycoproteins as contact cues in mate recognition. We expected a similar result to copepods, i.e. lectin-binding would reduce response of MP shrimp to lectin-treated newly molted EP shrimp. If the hypothesis is rejected, newly molted EP shrimp would be treated with ethylenediamine tetraacetate (EDTA) to remove glycoproteins on the cuticle surface, and response of MP shrimp to EDTA-treated EP shrimp would be observed. EDTA has been commonly used to extract glycoproteins (e.g. Andersen 1991; Shafer et al. 1994; Snell and Stelzer 2005).

Materials and methods

Experimental animals

Lysmata boggessi shrimp were collected from Hernando Beach, Florida, U.S.A., *L. wurdemanni* shrimp were collected at Sebastian Inlet, Sebastian, Florida, U.S.A., and *L. ankeri* shrimp were originally collected in Haiti. The MP and EP shrimp, between 2.6 and 3.8 cm in total length (TL), were maintained in 20-l tanks with flow-through seawater of 35‰ salinity and 26–28°C temperature, and were fed frozen adult *Artemia* ad libitum once daily.

Lectin binding and mate recognition

Lectin-binding experiments following the procedures of Kelly and Snell (1998) were performed to determine whether shrimp of the three *Lysmata* species use glycoproteins as contact chemical cues in mate recognition. Twenty lectins that have binding affinity with glycoproteins on exocuticle of decapod crustaceans (Marlowe et al. 1994) were screened. They belong to four affinity groups: glucose/mannose group, including agglutinin from *Pisum sativum* (PSA), *Lens culinaris* (LCA), and concanavalin A (Con A); *N*-acetyl glucosamine group including lectins from *Datura stramonium* (DSL), *Glycine max* (SBA), *Lycopersicon esculentum* (LEL), *Ricinus communis* (RCA), *Solanum tuberosum* (STL), lectin of *Vicia villosa* (VVL), *Triticum vulgaris* (WGA), and succinylated wheat germ agglutinin (SWGA); *N*-acetyl galactosamine/galactose group including lectins of *Bandeiraea* (*Griffonia*) *simplicifolia* (BSL I), *Dolichos biflorus* (DBA), *Erythrina corallodendron* (ECL), Jacalin (Jac), peanut agglutinin (*Arachis hypogaea*; PNA), *Sophora japonica* (SJA); and L-fucose group including *Ulex europaeus* (ULEX I); and oligosaccharides group including leucoagglutinin and erythroagglutinin of *Phaseolus vulgaris* (PHA-L, E). All lectins (lyophilized powder and fluoroisothiocyanate labeled) were purchased from Sigma Chemical Company or Vector Laboratories.

Because contact cues only exist in newly molted EP shrimp (NMEP) (Zhang and Lin 2006), lectin binding intensity by NMEP and intermolt EP (IEP) shrimp should be different if *Lysmata* shrimp use glycoproteins as their contact cues. Both IEP and NMEP shrimp were tested and compared. Result from the copepods indicates that lectin binding also inhibits male's performance in mate recognition (Kelly and Snell 1998). Hence, intermolt MP shrimp (IMP) was also treated with lectins to see whether lectin

binding affect MP shrimp's response to NMEP. Three concentrations, 10, 20, and 50 μg ml^{-1}, were pre-tested to determine the efficient binding concentration for the bioassay. Each lectin labeled with fluoroisothiocyanate (FITC) was used. All concentrations were effective, so the intermediate concentration (20 μg ml^{-1}) was used for the binding experiment. Three EP and MP shrimp were tested. For NMEP and IEP shrimp, exocuticle of each shrimp was dissected and cut into 21 pieces. Each was treated with one lectin of 20 μg ml^{-1} and seawater (control) for 60 min, then rinsed 5 times with seawater for about 3 min. (Because mating occurred within 2 min after NMEP shrimp were placed into the bucket containing IMP shrimp, see the Results). Previous result indicated that 30-min rinsing did not affect lectin binding (Marlowe et al. 1994). For IMP shrimp, only the antennae/antennules were tested, because IMP shrimp rely on two simple seta on antennae/antennules to detect the contact cues (Zhang and Lin 2006; Zhang et al. 2008). The treated exocuticles and the two simple setae on the antennae/antennules were observed under epifluorescent microscope. Microscope images were digitally captured, and the fluorescence can easily be quantified with the free program ImageJ. The binding affinities of the lectins were determined based on the difference in fluorescent readings between lectin-treated and control exocutiles or antennae/antennules. Lectin binding was graded based on relative staining intensities. Intense staining was noted as +++, moderate staining as ++, and weak staining as +. No mark indicates that the level of fluorescence was not different from that of the controls.

For bioassays, both MP and EP shrimp were treated with the lectins. Five to seven hours before EP shrimp molted (EP shrimp molt 12–24 h after larval hatching), they were moved from the maintenance tank and placed in a separate tank. The shrimp were checked every 30–60 min to determine the molting status. NMEP shrimp were exposed to 50 ml individual lectins at 20 μg ml^{-1} for 60 min. The experiments were performed with 10 MP and 10 EP shrimp of each species for each lectin. Control shrimp were exposed to seawater. The shrimp were re-used for different lectins test after at least one molting cycle (7–11 days).

Lectin-treated or non-lectin-treated EP shrimp were placed into 20-l buckets containing 10 l seawater with one lectin-incubated or with non-lectin-incubated MP shrimp to examine the response of MP shrimp to EP shrimp. MP shrimp (2.8 ± 0.2 cm, mean ± SD) were <0.5 cm smaller in total length than EP shrimp (3.1 ± 0.1 cm, mean ± SD), therefore would not affect mating success (Zhang and Lin 2005a). Environmental conditions (35‰ salinity and 26–28°C temperature) were the same as in the maintenance tanks. For control (non-lectin-treated MP and EP shrimp), MP shrimp would immediately grasp the EP shrimp and copulate with her after the MP shrimp had detected the EP shrimp with their antennae/antennules (Zhang and Lin 2005b, 2006). If glycoproteins were the contact cues, MP shrimp should not respond to lectin-treated EP shrimps or at least show a diminished response.

EDTA treatment and mate recognition

Because results from lectin binding experiment rejected the hypothesis that glycoproteins are contact cues of *Lysmata* shrimps (see Results), one of the three shrimp species, *L. boggessi*, was used to validate the lectin binding results. Newly molted EP shrimp were treated with 100 ml of 0.1 M EDTA at pH 7.5–8.0 for 30 min. EP shrimp were then rinsed with fresh EDTA solution, followed with seawater three times. Treated EP shrimp was placed into a 20-l bucket (containing 10 l seawater) containing 1 MP shrimp. Response of MP shrimp to EDTA-treated EP shrimp and control (non-EDTA treated newly molted EP shrimp) was observed and compared. Ten replicates were conducted.

Results

The patterns of binding intensity were the same among the three species (Table 1). All the lectins tested bound to the exocuticle of NMEP shrimp of all three species. The greatest binding was observed in the three lectins, Con A, Jac, and VVL. Most of these lectins bound only moderately or weakly to the exocuticle and two simple setae on antenna/antennules in all three species (Table 1).

Lectin treatment did not affect copulation success in the three *Lysmata* species (Fisher's exact test, $P = 1.000$), as all of the 10 MP shrimp tested in each species copulated successfully with lectin-treated and with control EP shrimp in each of the three treatments (non-lectin-treated MP and lectin-treated EP shrimp, lectin-treated MP and lectin-treated EP shrimp, and lectin-treated MP and non-lectin-treated EP shrimp). Responses of the MP shrimp in the three treatments and the control to lectin-treated and control EP shrimp were also not different for each species. When NMEP shrimp were placed into the bucket containing MP shrimp, the MP shrimp grasped the EP shrimp after detecting them with antennae/antennules within 2 min, and copulated with them regardless of the lectin treatment. As such all the 10 MP shrimp copulated with all 20 different lectin-treated and control EP shrimp successfully in each species.

EDTA treatment did not affect copulation success in *L. bogessi* (Fisher's exact test, $P = 1.000$). All 10 *L. boggessi* MP shrimp copulated with EDTA-treated newly molted shrimp and control newly molted shrimp immediately after they detected the EP shrimp with antennae/

Table 1 *Lysmata ankeri*, *Lysmata boggessi*, *Lysmata wurdemanni*

Lectin	NMEP	IEP	IMP ant
BSL I	+	+	+
CON A	+++	+++	++
DBA	+	+	+
DSL	++	+	+
ECL	+	+	+
Jac	+++	++	++
LCA	++	++	+
LEL	++	++	+
PHA-E	++	++	++
PHA-L	+	+	+
PNA	+	+	+
PSA	+	+	+
RCA	+	+	+
SBA	++	+	+
SJA	++	+	+
STL	+	+	+
SWGA	++	++	++
ULEX I	+	+	+
VVL	+++	+++	++
WGA	++	++	+
Control			

Lectin binding by exocuticle of newly molted (NMEP) and intermolt euhermaphroditic phase (IEP) shrimp, and antennae/antennules of intermolt male phase shrimp (IMP Ant). Lectin binding intensities among the three species were the same. Lectin abbreviations are the same as in "Materials and methods". Lectin binding was graded based on relative staining intensities. Intense staining was noted as +++, moderate staining as ++, and weak staining as +. No mark indicates that the level of fluorescence was not different from the controls

antennules. Interestingly, MP shrimp still copulated with six of the 10 EP shrimp killed by the EDTA.

Discussion

Since surface glycoproteins were discovered to function as mating pheromones in single-cell aquatic organisms such as algae (Wiese 1965) and ciliates (Miyake and Beyer 1974), these molecules have also been found in a rotifer (Snell et al. 1995) and in copepods to play a role in mate recognition (Snell and Carmona 1994; Kelly and Snell 1998; Ting et al. 2000). Although a number of aquatic organisms use surface glycoproteins as contact chemical recognition signals, lectin-binding experiments in the present study indicate that they are not contact pheromones for the *Lysmata* shrimps because MP shrimp of the three *Lysmata* species could recognize lectin-treated EP shrimp and copulated with them. EDTA treatment test further validated the results of lectin binding experiment.

In the harpacticoid copepod *Coullana canadensis*, mate guarding was reduced by binding lectins to female surface glycoproteins (Lonsdale et al. 1996). Glycoproteins also exist on cuticle surface of decapod crustaceans (Marlowe et al. 1994); however, binding lectins to surface glycoproteins of NMEP shrimp did not reduce responses of MP shrimp to the treated EP shrimp and did not affect copulation success. This suggests that glycoproteins are not likely involved in mate recognition in the *Lysmata* shrimps. On the other hand, lectins may also lower sensitivity of male copepods' chemoreceptor, so that male mate-guarding is significantly decreased (Kelly and Snell 1998). Although lectins also bound on two simple setae on antennae/antennules, which are probably the detectors of contact cues in *Lysmata* shrimps, male's responses to NMEP were not reduced.

EDTA has been used in extracting glycoproteins from cuticles of crustaceans (e.g. Andersen 1991; Shafer et al. 1994). Removal of glycoproteins in female rotifers with EDTA incubation significantly reduced mating responses from males (Snell and Stelzer 2005). No difference in copulation response from MP shrimp to EDTA-treated and control EP shrimp in the present study further validates that glycoproteins are not likely involved in mate recognition in the *Lysmata* shrimps.

Chemical components of sexual pheromone bouquets often exist in one sex and are absent in the other (e.g. Wabnitz et al. 2000; Shine et al. 2002; Zhang et al. 2003), most prominently shown in sex specific attractants in insects but also in crustaceans. For *Lysmata* shrimps, there maybe different contact cues for different molt stages because MP shrimp only mate with NMEP shrimp. Active components should exist on the exocuticle of the newly molted animals, and absent or in very low concentrations in the other stages of the molt cycle. Studies have shown that the surface glycoprotein composition of decapod crustaceans changes during the molt cycle (Marlowe et al. 1994; Shafer et al. 1994), but lectin binding results indicate that glycoprotein concentrations on the exocuticle of newly molted decapod crustaceans are not different from those in other molt stages (Marlowe et al. 1994). Our study shows that lectin binding intensities were similar between NMEP and IEP (Table 1), further suggesting that glycoproteins are not likely involved in mate recognition in the *Lysmata* shrimps.

The reason *Lysmata* shrimps do not use glycoproteins as contact chemical cues may be the insufficient variation in glycoprotein composition on the exocuticle at different molt stages. Contact pheromones have been intensively studied in insects and have been identified as cuticular hydrocarbons, not glycoproteins (e.g. Linn and Roelofs 1995; Ginzel et al. 2003; Howard et al. 2003; Zhang et al. 2003; Sugeno et al. 2006). Future studies on *Lysmata*

shrimps will examine whether their contact pheromones are hydrocarbons and attempt to chemically characterize the contact sex pheromones.

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References

Ameyaw-Akumfi C, Hazlett BA (1975) Sex recognition in crayfish *Procambarus clarkii*. Science 190:1225–1226

Andersen SO (1991) Cuticular proteins from the shrimp, *Pandalus borealis*. Comp Biochem Physiol B 99:453–458

Atema J (1984) Review of sexual selection and chemical communication in the lobster, *Homarus americanus*. Can J Fish Aquat Sci 43:2283–2290

Bauer RT (1979) Sex attraction and recognition in the caridean shrimp *Heptacarpus paludicola* Holmes (Decapoda: Hippolytidae). Mar Behav Physiol 6:157–174

Bauer RT (2000) Simultaneous hermaphroditism in caridean shrimps: a unique and puzzling sexual system in the Decapoda. J Crust Biol 20:116–128

Burkenroad MD (1947) Reproductive activities of decapod crustacean. Am Nat 81:392–398

Caskey JL, Bauer RT (2005) Behavioral tests for a possible contact sex pheromone in the caridean shrimp *Palaemonetes pugio*. J Crust Biol 25:571–576

Dunham PJ (1978) Sex pheromones in Crustacea. Biol Rev 53: 555–583

Dunham PJ (1988) Pheromones and behavior in Crustacea. In: Laufer H, Downer GH (eds) Endocrinology of selected invertebrate types. Alan R. Liss, New York, pp 375–392

Etges WJ, Jackson LL (2001) Epicuticular hydrocarbon variation in *Drosophila mojavensis* cluster species. J Chem Ecol 27: 2125–2149

Fletcher N, Hardege JD (2009) The cost of conflict: agonistic encounters influence responses to chemical signals in the shore crab, *Carcinus maenas*. Anim Behav 77:357–361

Focarelli R, Rosati F (1995) The 220-kDa vitelline coat glycoprotein mediated sperm binding in the polarized egg of *Unio elongatulus* through O-linked oligosaccharides. Develop Biol 171:606–614

Ginzel MD, Blomquist GJ, Millar JG, Hanks LM (2003) Role of contact pheromones in mate recognition in *Xylotrechus colonus*. J Chem Ecol 29:533–545

Glabe CG, Clark D (1991) The sequence of the *Arbacia punctulata* Bindin cDNA and implications for the structural basis of species-specific sperm adhesion and fertilization. Develop Biol 143: 282–288

Gleeson RA (1980) Pheromone communication in the reproductive behavior of the crab, *Callinectes sapidus*. Mar Behav Physiol 7:119–134

Hardege JD, Jennings A, Hayden D, Müller CT, Pascore D, Bentley MG, Clare AS (2002) Novel behavioural assay and partial purification of a female-derived sex pheromone in *Carcinus maenas*. Mar Ecol Prog Ser 244:179–189

Higgie M, Chenoweth S, Blows MW (2000) Natural selection and the reinforcement of mate recognition. Science 290:519–521

Howard RW, Jackson LL, Banse H, Blows MW (2003) Cuticular hydrocarbons of *Drosophila birchii* and *D. serrata* identification and role in mate choice. J Chem Ecol 29:961–976

Kamiguchi Y (1972) Mating behavior in the freshwater prawn, *Palaemon paucidens*. A study of the sex pheromone and its effect on males. J Facult Sci Hokkaido Univ, Ser VI, Zool 18:347–355

Kelly LS, Snell TW (1998) Role of surface glycoproteins in mate-guarding of the marine harpacticoid *Tigriopus japonicus*. Mar Biol 130:605–612

Linn CE, Roelofs WL (1995) Pheromone communication in moths and its role in the speciation process. In: Lambert DM, Spencer HG (eds) Speciation and the recognition concept: theory and application. The Johns Hopkins University Press, Baltimore and London, pp 263–300

Lonsdale DJ, Snell TW, Frey MA (1996) Lectin binding to surface glycoproteins on *Coullana* spp. (Copepoda: Harpacticoida) can inhibit mate guarding. Mar Behav Physiol 27:153–162

Lopez A, Miraglia SJ, Glabe CG (1993) Structure/function analysis of the sea urchin sperm adhesive protein binding. Develop Biol 156:24–33

Marlowe RL, Dillaman RM, Roer RD (1994) Lectin binding by crustacean cuticle: the cuticle of *Callinectes sapidus* throughout the molt cycle, and the intermolt cuticle of *Procambarus clarkii* and *Ocypode quadrata*. J Crust Biol 14:231–246

Miyake A, Beyer J (1974) Blepharmone: a conjugation-inducing glycoprotein in the ciliate *Blepharisma*. Science 185:621–623

Ryan EP (1966) Pheromone: evidence in a decapod crustacean. Science 151:340–341

Seifert P (1982) Studies on the sex pheromone of the shore crab, *Carcinus maenas*, with special regard to ecdysone excretion. Ophelia 21:147–158

Shafer TH, Roer RD, Miller CG, Dillaman RM (1994) Postecdysial changes in the protein and glycoprotein composition of the cuticle of the blue crab *Callinectes sapidus*. J Crust Biol 14:210–219

Shine RR, Reed N, Shetty S, Lemaster M, Mason RT (2002) Reproductive isolating mechanisms between two sympatric sibling species of sea snakes. Evolution 56:1655–1662

Snell TW, Carmona MJ (1994) Surface glycoproteins in copepods: potential signals for mate recognition. Hydrobiologia 292(293): 255–264

Snell TW, Stelzer CP (2005) Removal of surface glycoproteins and transfer among *Brachionus* species. Hydrobiologia 546:267–274

Snell TW, Rico-Martinez R, Kelly LS, Battle TE (1995) Identification of a sex pheromone from a rotifer. Mar Biol 123:347–353

Sugeno W, Hori M, Matsuda K (2006) Identification of the contact sex pheromone of *Gastrophysa atrocyanea* (Coleoptera: Chrysomelidae). Appl Entomol Zool 41:269–276

Tierney AJ, Thompson CS, Dunham EW (1984) Site of pheromone reception in the crayfish *Orconectes propinquus* (Decapoda, Cambaridae). J Crust Biol 4:554–559

Ting JH, Kelly LS, Snell TW (2000) Identification of sex, age and species-specific proteins on the surface of the harpacticoid copepod *Tigriopus japonicus*. Mar Biol 137:31–37

Wabnitz PA, Bowie JH, Tyler MJ, Wallace JC, Smith BP (2000) Differences in the skin peptides of the male and female Australian tree frog *Litoria splendida*. Eur J Biochem 267: 269–275

Wiese L (1965) On sexual agglutination and mating type substances (gamones) in isogamous heterophallic Chlamydamondes. I. Evidence of the identity of the gamone with the surface components responsible for sexual flagellar content. J Phycol 1:46–54

Zhang D, Lin J (2005a) Comparative mating success of smaller male-phase and larger male-role euhermaphrodite-phase shrimp, *Lysmata wurdemanni* (Caridea: Hippolytidae). Mar Biol 147: 1387–1392

Zhang D, Lin J (2005b) Mating without anterior pleopods in shrimp *Lysmata wurdemanni*, a protandric simultaneous hermaphrodite (Crustacea: Decapoda: Caridea). Crustaceana 77:1203–1212

Zhang D, Lin J (2006) Mate recognition in a simultaneous hermaphroditic shrimp, *Lysmata wurdemanni* (Caridea: Hippolytidae). Anim Behav 71:1191–1196

Zhang A, Oliver JE, Chauhan K, Zhao B, Xia L, Xu Z (2003) Evidence for contact sex recognition pheromone of the Asian longhorned beetle, *Anoplophora glabripennis* (Coleoptera: Cerambycidae). Naturwissenschaften 90:410–413

Zhang D, Cai S-L, Liu H, Lin J (2008) Antennal sensilla in the genus *Lysmata* (Caridea). J Crust Biol 28:433–438