

## EVOLUTION OF WAVING DISPLAY IN BRACHYURAN CRABS OF THE GENUS *ILYOPLAX*

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### ABSTRACT

Male waving displays of the species of *Ilyoplax* (Brachyura: Ocypodidae) have so far been described in *I. delsmanni*, *I. gangetica*, and *I. orientalis*. In this study, male waving displays of *I. dentata*, *I. dentimerosa*, *I. deschampsi*, *I. formosensis*, *I. integra*, *I. ningpoensis*, *I. pingi*, *I. pusilla*, *I. serrata*, *I. strigicarpa*, *I. tansuiensis*, and *Ilyoplax* sp. are described for the first time. Waving display of the above 15 species of *Ilyoplax* can be classified into three patterns; a circular type, vertical type, and asymmetrical type. Asymmetrical wave forms were observed in only two species, *I. orientalis* and *I. tansuiensis*, vertical wave forms were seen in four species, *I. delsmanni*, *I. dentimerosa*, *I. pingi*, and *I. serrata*, while most other species exhibited circular wave forms. To understand the evolutionary divergence of these waving forms observed in species of *Ilyoplax*, a molecular phylogeny was reconstructed based on 1005-bp nucleotide sequences from mitochondrial 16S and 12S rRNA genes of 16 species and two out-group taxa. Phylogenetic analysis of waving forms suggested that circular waves evolved once in the early history of the lineage. Vertical wave forms were suggested to have subsequently evolved three times independently from circular wave forms, and the asymmetrical wave form has also evolved from circular wave form. The evolution of distinct waving forms is considered to be associated with the presence of geographic sympatric species.

### INTRODUCTION

Many species of grapsoid and ocypodoid male crabs are known to display waving, during which they move their body rhythmically whilst the chelipeds are raised and lowered. Waving in some ocypodoids is considered to have both a courtship as well as agonistic functions (Salmon, 1965, 1984; Crane, 1975; Wada, 1981, 1984, 1993; Moriito and Wada, 1997), and the motion is variable at the species level (Crane, 1975; Kitaura and Wada, 2004). This diversity in waving displays may have arisen as a consequence of biological (presence of sympatric congeneric species), and/or physical factors in the environment of each species. However, relationships between these factors and waving form have not yet been examined.

The genus *Ilyoplax* Stimpson, 1858 (Ocypodidae, Dotillinae), contains about 25 species that are common inhabitants of intertidal mud flats from the temperate to tropical Indo-West Pacific region. Several types of waving motion in the genus *Ilyoplax* have been reported (Tweedie, 1954; Nishihira, 1984; Kosuge et al., 1994; Wada et al., 1996). Waving in some species of *Ilyoplax* is known to have a courtship function (Kosuge et al., 1994), as reported in other species of ocypodid (Wada, 1984). To understand the evolution of waving form divergence in *Ilyoplax*, it is necessary to understand phylogenetic relationships among the species. In a previous study, we used mtDNA molecular characters to characterize the phylogenetic relationships among 11 species of *Ilyoplax* (Kitaura et al., 1998). In this study, we determined partial sequences of 12S and 16S rRNA genes for more six species of *Ilyoplax*, clarifying phylogenetic relationships among a total of 17 species. Waving form data gathered from field observations and the literature were subsequently mapped on the molecular phylogeny to reconstruct the evolutionary history of their waving form. In addition, the evolution of waving form was

examined relative to the co-occurrence of congeneric species.

### MATERIALS AND METHODS

*Ilyoplax* were collected for mtDNA sequencing from the following locations: *Ilyoplax delsmanni*, *I. gangetica*, *I. obliqua*, and *I. orientalis*: Ranong, Thailand, 12/9/1999; *I. punctata*: Can Gio, Vietnam, 7/27/2000; *Ilyoplax* sp.: Lucap, Alaminos Prov., Philippines, 12/14/2000. These data were then compared to comparable data obtained previously from the following 12 species: *Ilyoplax dentata*, *I. dentimerosa*, *I. deschampsi*, *I. formosensis*, *I. ningpoensis*, *I. pingi*, *I. pusilla*, *I. serrata*, *I. strigicarpa*, *I. tansuiensis*, and two out-group taxa *Ilyoplax integra* and *Scopimera globosa*; Kitaura et al., 1998; genetic database accession No. AB002113-AB002124). The data set (Table 1) include 17 of the 25 species of the *Ilyoplax* that occur in the Indo-West Pacific. Two out-groups were chosen according to the phylogenetic hypothesis proposed in Kitaura et al. (1998).

Total genomic DNA was extracted from the abdomen or ambulatory leg musculature of each crab using the QIAamp Tissue Kit (QIAGEN). Selective amplification of portions of the 16S and 12S mitochondrial rRNA genes was carried out by polymerase chain reaction (PCR), using the primers L1496i (5'-GTACATATCGCCCGTCGCTT-3') (Kitaura et al., 1998), L2510C (5'-CGCCTGTTTAAACAAAGACAT-3') (modified from Palumbi et al., 1991), H2492i (5'-CAGACATGTTTTAATAAACAGGC-3') (modified from Palumbi et al., 1991), H2716i (5'-AAGTTTTA-TAGGGTCTTATCGTC-3') (Kitaura et al., 1998), H3058 (5'-TCCGG-TCTGAACTCAGATCAGTA-3') (Kitaura et al., 1998), H3059 (5'-CCGGTCTGAACTCAGATCAGT-3') (Palumbi et al., 1991) and H3062 (5'-CCGGTCTGAACTCAGATCA-3') (modified from Palumbi et al., 1991) (PCR conditions: 30–35 cycles of 40 sec 94°C/1 min 72°C denaturing/annealing/extension temperatures).

Amplification products were purified using the QIAquick purification kit (QIAGEN). These purified products were directly sequenced using the ABI BigDye terminator mix with an ABI Prism 310 Genetic Analyzer. All final sequences were obtained from both strands for verification. Sequence from one individual was determined for each species studied. All nucleotide sequences were submitted to the DDBJ nucleotide sequences databases under accession numbers AB234873-AB234878.

The DNA sequences were initially aligned using CLUSTAL W (Thompson et al., 1994) with default gap penalties, with small subsequent modifications made by eye using the sequence-analysis software SeqPup 0.6 (Gilbert, 1996). Positions that could not be aligned with certainty were excluded from the data sets.

Table 1. Waving observation localities and dates.

Species	Observation locality	Observation date	Observer
<i>I. delsmanni</i> de Man, 1926	Ranong, Thailand	23-24/Nov/1982, 9/Dec/1999	JK,KW
<i>I. dentata</i> Ward, 1933	Ayton, Australia	30/Nov/1994	KW
<i>I. dentimerosa</i> Shen, 1932	Kanghwa Is., Korea	19/May/1992	KW
<i>I. deschampsi</i> (Rathbun, 1913)	Ohkawa, Fukuoka Pref., Japan	15-16/July/1993	KW
<i>I. formosensis</i> (Rathbun, 1921)	Tungshi, Taiwan	12-13/Mar/1996	KW
	Giao Thuy, Vietnam	20-21/July/2001	JK,KW
<i>I. gangetica</i> Kemp, 1919	Ranong, Thailand	23-24/Nov/1982, 6/Dec/1999	JK,KW
<i>I. ningpoensis</i> Shen, 1940	Haiphong, Vietnam	6/Apr/1995	KW
	Giao Thuy, Vietnam	20-21/July/2000	JK,KW
<i>I. obliqua</i> Tweedie, 1935	Ranong, Thailand	16-19/Dec/1982, 9/Dec/1999	JK,KW
<i>I. pingi</i> Shen, 1932	Kanghwa Is., Korea	19/May/1992	KW
<i>I. punctata</i> Tweedie, 1935	Can Gio, Vietnam	25,27/July/2000	JK,KW
<i>I. pusilla</i> (de Haan, 1835)	Shirahama, Wakayama Pref., Japan	20-29/June/1994	JK
<i>I. serrata</i> Shen, 1931	Giao Thuy, Vietnam	20-21/July/2000	JK,KW
	Can Gio, Vietnam	27/July/2001	JK,KW
<i>I. strigicarpa</i> Davie, 1988	Townsville, Australia	3/Dec/1994	KW
	Cairns, Australia	4/Dec/1994	KW
	Brisbane, Australia	12/Dec/1994	KW
	Darwin, Australia	25/Aug-1/Sep,1994	KW
<i>I. tansuiensis</i> Sakai, 1939	Tanshui, Taiwan	29/May/1992	KW
<i>Ilyoplax</i> sp.	Luzon Is., the Philippines	14/Dec/2000	JK,KW
Out-group			
<i>Ilyoplax integra</i> (Tesch, 1918)	Okinawa Is., Okinawa Pref., Japan	2/Apr/1987, 12-15, 29-31/July/1998	JK,KW

Phylogenetic relationships were analyzed using three major phylogenetic procedures: PAUP\* ver. 4.0b8 (Swofford, 1998): maximum likelihood (ML), maximum parsimony (MP) and neighbor joining (NJ) methods. A MP tree was constructed with a heuristic search algorithm with 10 replications of random stepwise additions. All other parameters used were those of the default options of the software. Gaps were always treated as missing. To evaluate the robustness of the internal branches, 1000 bootstrap

replications (Felsenstein, 1985) were performed, using a heuristic search with random sequence addition.

We calculated the model of DNA substitution that best fitted our data using the software MODELTEST 3.06 (Posada and Crandall, 1998). The suggested model of DNA evolution was subsequently used to infer phylogenetic relationships with ML and NJ methods. ML analyses were performed using heuristic search algorithms, and setting parameters to values calculated by

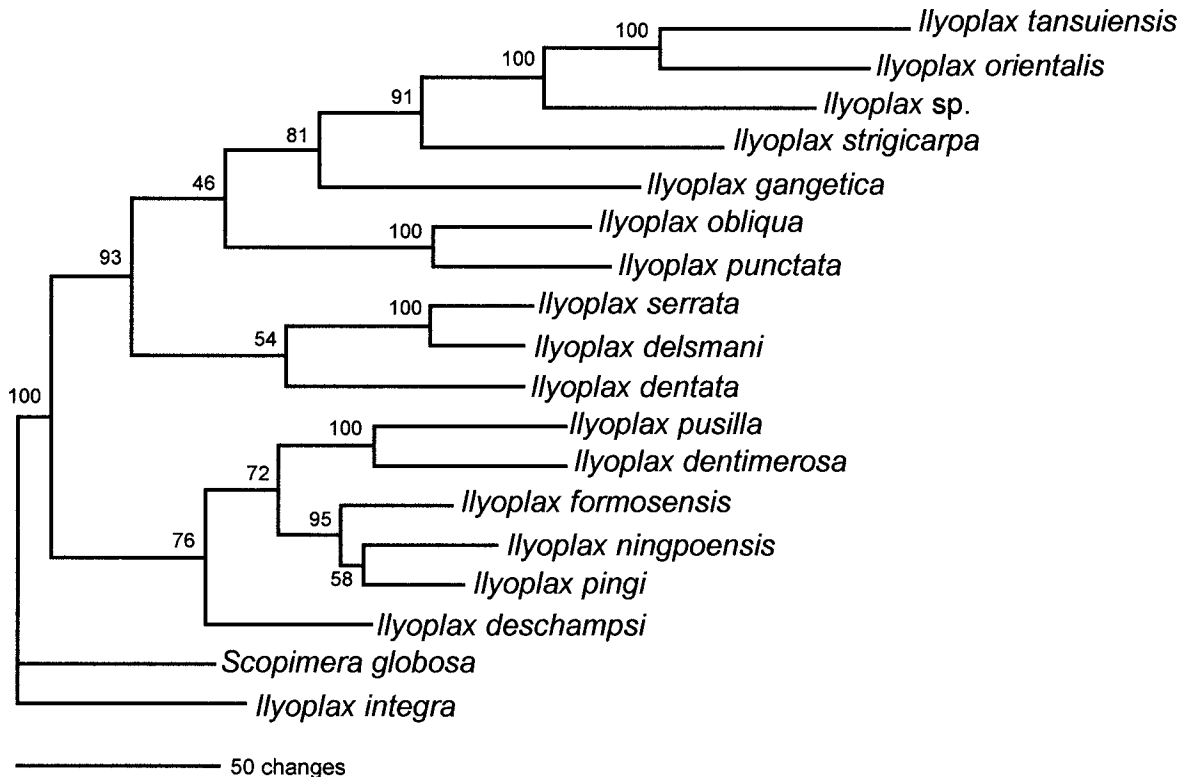


Fig. 1. The single most-parsimonious tree for 16 species of *Ilyoplax* and out-group taxa based on 1005 base pairs of the 16S and 12S mitochondrial rRNA genes. *Scopimera globosa* and *Ilyoplax integra* were used as out-groups. The numbers at each internal branch indicates the percentages of bootstrap values from 1000 replicates.

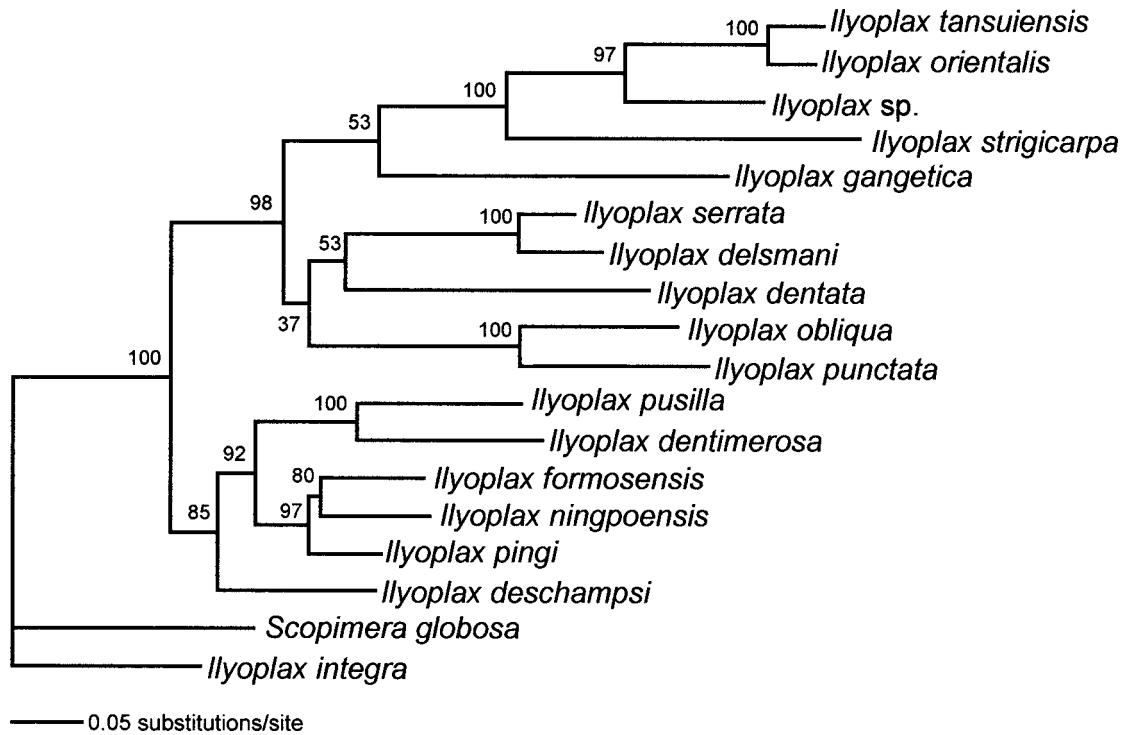


Fig. 2. Maximum likelihood tree for 16 species of *Ilyoplax* and outgroup taxa using the TVM + I + G distance model. *Scopimera globosa* and *Ilyoplax integra* were used as out-groups. Numbers are bootstrap values from 100 replicates.

MODELTEST. For both methods, bootstrap analyses as a heuristic search were applied, with 100 replications for ML and 1000 replications for NJ.

Waving displays for each species were recorded using a video camera during low tide (see Table 1 for localities and observation dates). Waving forms for each species (see above) were superimposed on the inferred tree by using the unordered parsimony principle (MacClade version, 3.08; Maddison and Maddison, 1992). These examined behavioral characters were treated as genetically controlled characters and, when observed in the field, a character was regarded as being possessed obligatorily for the relevant species without intraspecific variation. All state changes were

considered to be equally probable, and all possible optimizations were examined using equivocal cycling option in MacClade.

## RESULTS

### Phylogenetic Relationships

The total alignments of the sequenced portions of mitochondrial 16S and 12S ribosomal DNA region consisted of 1321 base pairs. Of the aligned sequences, hypervariable regions

Table 2. Characteristics of waving display in each species of *Ilyoplax*.

Species	Waving forms		Other elements				Source
	Circular (C)/Vertical (V)/Asymmetrical (A)	Forward/Non-Forward	Pause at maximum elevation	Speed changing	Chela quivering		
<b>Genus <i>Ilyoplax</i></b>							
<i>I. dentata</i> Ward, 1933	C	—	no	no	yes	present study	
<i>I. deschampsi</i> (Rathbun, 1913)	C	—	no	yes	no	present study	
<i>I. formosensis</i> (Rathbun, 1921)	C	—	no	no	no	present study	
<i>I. gangetica</i> Kemp, 1919	C	—	no	no	yes	Kosuge et al. (1994); present study	
<i>I. ningpoensis</i> Shen, 1940	C	—	no	no	no	present study	
<i>I. pusilla</i> (de Haan, 1835)	C	—	no	no	no	present study	
<i>I. strigicarpa</i> Davie, 1988	C	—	no	no	yes	present study	
<i>Ilyoplax</i> sp.	C	—	no	no	yes	present study	
<i>I. delsmeni</i> de Man, 1926	V	F	no	no	yes	Kosuge et al. (1994); present study	
<i>I. dentimerosa</i> Shen, 1932	V	NF	yes	no	no	present study	
<i>I. pingi</i> Shen, 1932	V	NF	no	no	no	present study	
<i>I. serrata</i> Shen, 1931	V	F	no	no	yes	present study	
<i>I. orientalis</i> (de Man, 1888)	A	—	no	no	yes	Kosuge et al. (1994)	
<i>I. tansuiensis</i> Sakai, 1939	A	—	no	no	yes	present study	
<i>I. obliqua</i> Tweedie, 1935	Absent	—	—	—	yes	present study	
<i>I. punctata</i> Tweedie, 1935	Absent?	—	—	—	no?	present study	
<b>Out-group</b>							
<i>Ilyoplax integra</i> (Tesch, 1918)	C	—	no	no	no	present study	
<i>Scopimera globosa</i> (de Haan, 1835)	C	—	no	yes	no	Moriito and Wada (1997)	

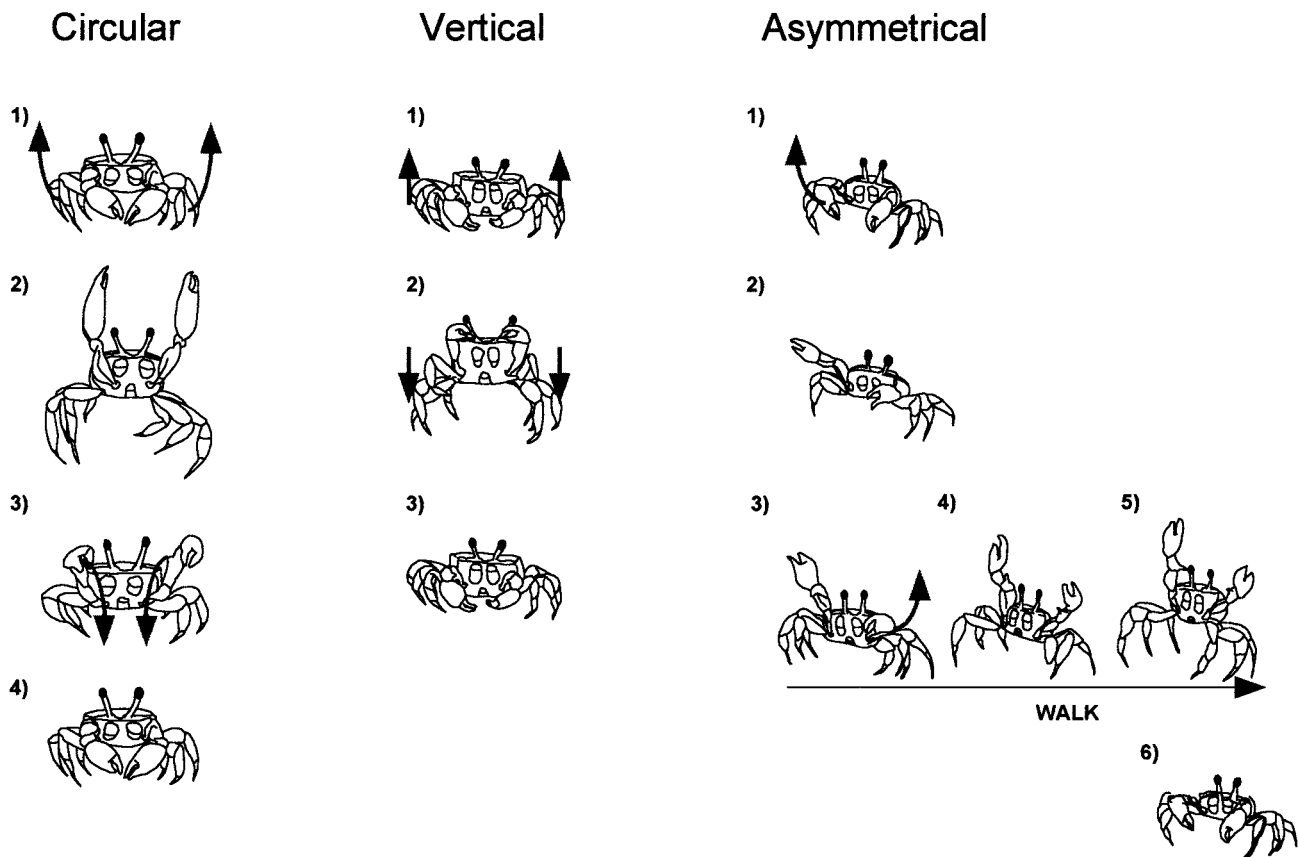


Fig. 3. Three typical waving patterns in species of *Ilyoplax*. Arrows indicate wave track.

that could not be aligned unambiguously were discarded from the analysis resulting in a total of 1005 base pairs, which were used for phylogenetic analysis. These included 515 variable and 382 phylogenetically informative sites.

The MP heuristic search yielded a single most-parsimonious tree, with a tree length of 1517 (consistency index [CI]=0.534, retention index [RI]=0.478, and rescaled consistency index [RC]=0.255). The topology of this search was identical to the topology obtained with the bootstrap method (Fig. 1).

Using Modeltest (Posada and Crandall, 1998), the best-fitting model of substitutions was the TVM + I + G model with the following parameter values: base frequencies: A = 0.4021, C = 0.0793, G = 0.1220, T = 0.3966; substitutions model rate matrix; [A-C]=0.5361, [A-G]=6.2961, [A-T]=0.6663, [C-G]=0.8418, [C-T]=6.2961, [G-T]=1.0000; proportion of invariable sites = 0.3205, gamma shape parameter = 0.6806. The NJ analysis produced the same topology as that found by the MP analysis, except for the placement of *I. formosensis* and *I. ningpoensis*. The ML tree also showed almost the same topology as that found by the MP, differing only in the placement of *I. formosensis* and *I. ningpoensis* and of the cluster constituting *I. obliqua* and *I. punctata* (Fig. 2).

#### Waving Display

Waving display of *Ilyoplax* species have so far been described on *I. delsmanni*, *I. gangetica* and *I. orientalis* (Kosuge et al., 1994). In this study, waving displays of 12 additional species *I. dentata*, *I. dentimerosa*, *I. deschampsii*, *I. formosensis*,

*I. integra*, *I. ningpoensis*, *I. pingi*, *I. pusilla*, *I. serrata*, *I. strigicarpa*, *I. tansuiensis*, and *Ilyoplax* sp. are described and classified for the first time (Table 2). No waving displays were observed in two species (*I. obliqua* and *I. punctata*). But *I. punctata* was treated as a behavioral unknown species in the trait mapping because the observation (only one-day) was not enough to ensure the absence of waving.

Waving motions were classified into three patterns, defined as follows (Fig. 3): asymmetrical: at the start, both chelipeds are flexed in front of the buccal region, then one cheliped is abruptly raised, unflexed vertically, then stopped pointing upward. Soon after the first cheliped moves, the other is unflexed laterally, then raised. Both chelipeds are then lowered (2 species); circular: both chelipeds are unflexed forward-laterally, raised until the tips point upwards, then both chelipeds are lowered vertically. Both chelipeds move a lateral to a medial position and the tips of chela describe a semicircle arc (9 species); vertical: both chelipeds are raised vertically upward above the carapace, then are lowered back to their initial position (4 species; Table 2).

Fig. 5. Chela quivering behavior mapped parsimoniously on the inferred tree (Fig. 1) for the species of *Ilyoplax*. Ancestral states are indicated with patterned branches that match patterns in terminal branches. Branches leading to the out-groups *Scopimera globosa* and *Ilyoplax integra* are deleted from the figure.

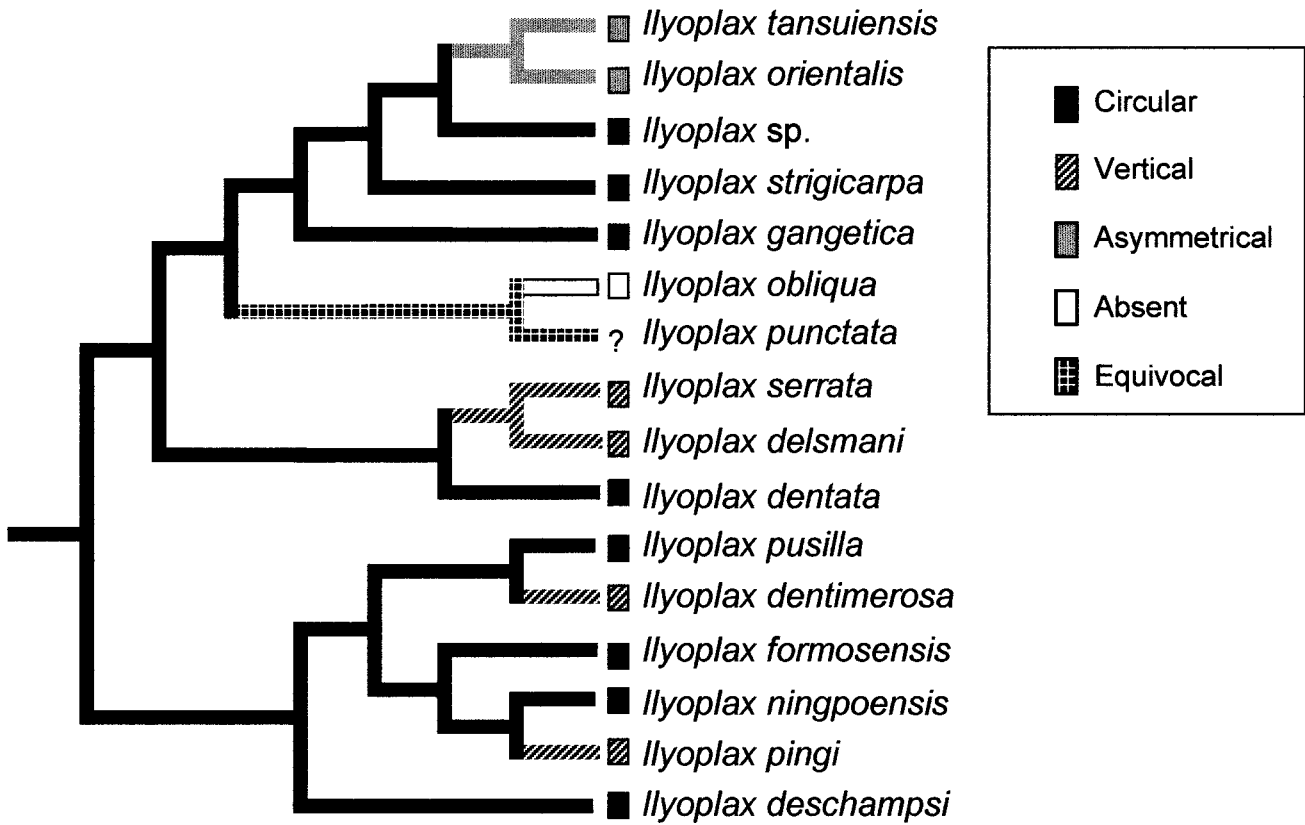


Fig. 4. Waving forms mapped parsimoniously on the inferred tree (Fig. 1) for species of *Ilyoplax*. Ancestral states are indicated with patterned branches that match patterns in terminal branches. Branches leading to the out-groups *Scopimera globosa* and *Ilyoplax integra* are deleted from the figure.

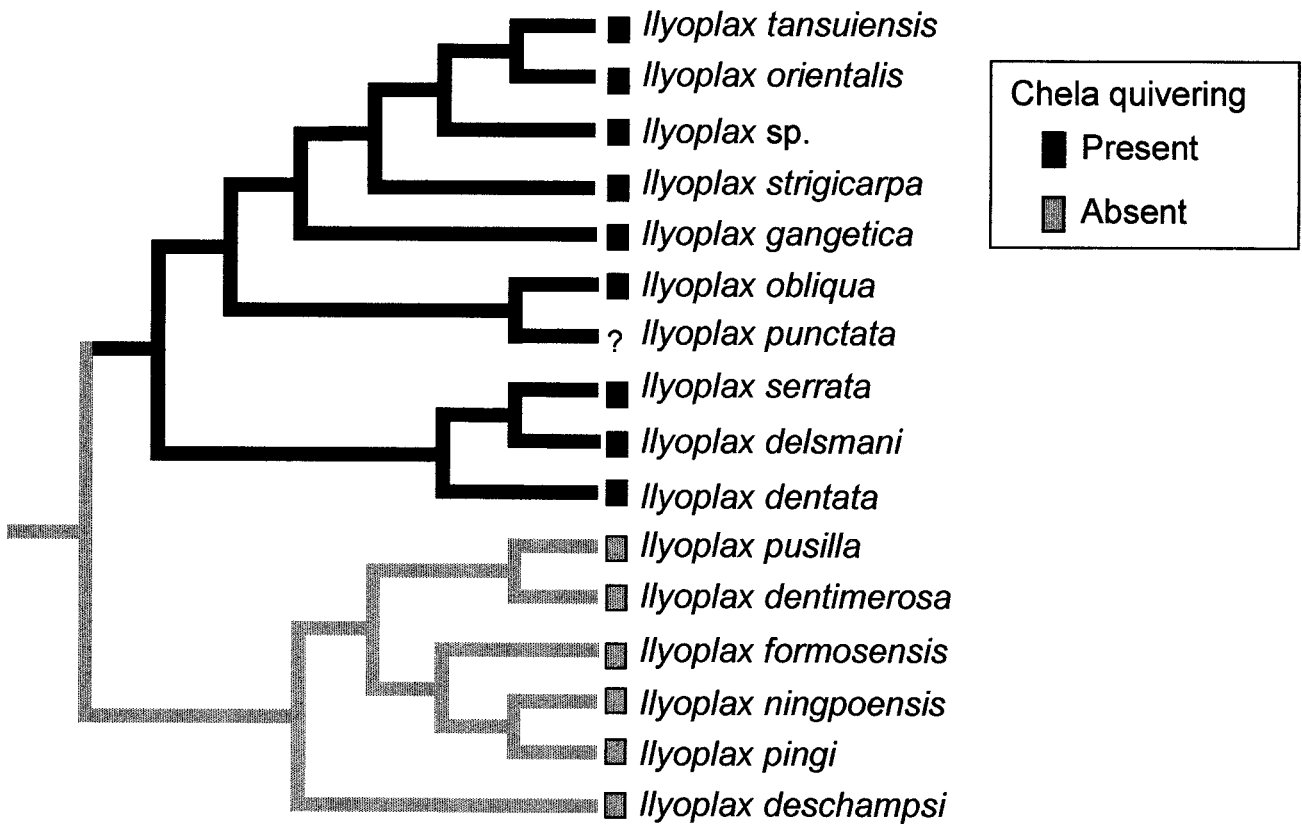


Table 3. Geographic distribution and habitat condition for each species of *Ilyoplax*. Sources, denoted by superscript numerals, are as follows: <sup>1</sup> Davie (1988) and Wada (unpublished data); <sup>2</sup> Rathbun (1913), Shen (1932), Kamita (1941), Kim and Kim (1982), Dai and Yang (1991), and Wada et al. (1992); <sup>3</sup> Gao and Li (1985), Dai and Yang (2001), and Kosuge et al. (1997); <sup>4</sup> Kemp (1919), Tweedie (1937), Kosuge et al. (1994), and Wada (unpublished data); <sup>5</sup> Dai and Yang (1991), and Kosuge et al. (1997); <sup>6</sup> Wada et al. (1992); <sup>7</sup> Kitaura and Wada (unpublished data); <sup>8</sup> Tweedie (1935;1937), Lundoer (1974), Serene and Lundoer (1974), and Tan and Ng (1994); <sup>9</sup> Shen (1932), Kamita (1941), Kim and Kim (1982), and Dai and Yang (1991); <sup>10</sup> Kamita (1941), Kim and Kim (1982), and Dai and Yang (1991); <sup>11</sup> Shen (1931), Tweedie (1935;1950), Serene and Lundoer (1974), Gao and Li (1985), Dai and Yang (1991), and Wada (unpublished data); <sup>12</sup> Kemp (1919), Tweedie (1935; 1950), Lundoer (1974), and Tan and Ng (1994); <sup>13</sup> Sakai (1939), Gao and Li (1985), Dai and Yang (1991), Fukui et al. (1989), and Kitaura and Wada (unpublished data); <sup>14</sup> Tweedie (1935), Lundoer (1974), and Tan and Ng (1994); <sup>15</sup> Tweedie (1935), Tan and Ng (1994), and Kitaura and Wada (unpublished data); <sup>16</sup> Kim et al. (1981), and Wada et al. (1992); <sup>17</sup> Kosuge et al. (1997), and Wada and Wang (1998); <sup>18</sup> Kemp (1919), Tweedie (1935) and Kosuge et al. (1994); <sup>19</sup> Kosuge et al. (1997); <sup>20</sup> Ono (1965), Wada and Tsuchiya (1975), Wada (1976), and Wada et al. (1992); <sup>21</sup> Tweedie (1935), Sasekumar (1974), Frith et al. (1976), Kosuge et al. (1994), and Kitaura and Wada (unpublished data); <sup>22</sup> Kim et al. (1981), Koh and Shin (1988), and Wada (unpublished data); <sup>23</sup> Koh and Shin (1988), and Wada (unpublished data); <sup>24</sup> Kosuge et al. (1994), and Wada (unpublished data); <sup>25</sup> Tweedie (1935), Sasekumar (1974), and Kosuge et al. (1994); <sup>26</sup> Wada and Wang (1998), and Wada (unpublished data); <sup>27</sup> Sasekumar (1974), Frith et al. (1976), and Wada (unpublished data).

Species	Geographic distribution	Intertidal habitat
<i>I. dentata</i>	Eastern Australia <sup>1</sup>	Mud banks of mid to upper estuary <sup>1</sup>
<i>I. deschampsii</i>	Ariake Sea (Japan), northern China, western coast of Korea <sup>2</sup>	Soft mud flats of mid to upper estuary <sup>16</sup>
<i>I. formosensis</i>	Taiwan, southern China, northern Vietnam <sup>3</sup>	Mud flats of mid to upper estuary <sup>17</sup>
<i>I. gangetica</i>	Ganges River Delta, west coast of Malay Peninsula <sup>4</sup>	Mud flats of estuary and sheltered bay <sup>18</sup>
<i>I. ningpoensis</i>	Southern China, northern Vietnam <sup>5</sup>	Mud flats at the mouth of estuary <sup>19</sup>
<i>I. pusilla</i>	Japan, southern coast of Korea <sup>6</sup>	Sandy mud/mud flats of estuary <sup>20</sup>
<i>I. strigicarpa</i>	Northern Australia <sup>1</sup>	Mud flats at the mouth of estuary and sheltered bay <sup>1</sup>
<i>Ilyoplax</i> sp.	Luzon Is. (the Philippines), Celebes (Indonesia) <sup>7</sup>	Mud flats of sheltered bay <sup>7</sup>
<i>I. delsmanni</i>	Djakarta Bay (Indonesia), Singapore, west coast of Malay Peninsula <sup>8</sup>	Mud flats/banks of estuary and sheltered bay <sup>21</sup>
<i>I. dentimerosa</i>	Northern China, western coast of Korea <sup>9</sup>	Firm mud flats of lower to mid estuary <sup>22</sup>
<i>I. pingi</i>	Northern China, western coast of Korea <sup>10</sup>	Mud flats of lower to mid estuary <sup>23</sup>
<i>I. serrata</i>	Southern China, Vietnam, east coast of Malay Peninsula, Sarawak (Malaysia) <sup>11</sup>	Mud flats at the mouths of estuary <sup>24</sup>
<i>I. orientalis</i>	Malay Peninsula, Sarawak (Malaysia) <sup>12</sup>	Mud flats of estuary and sheltered bay <sup>25</sup>
<i>I. tansuiensis</i>	Taiwan, southern China, Vietnam <sup>13</sup>	Mud flats of lower to mid estuary <sup>26</sup>
<i>I. obliqua</i>	Singapore, west coast of Malay Peninsula <sup>14</sup>	Forested/foreshore mud flats/banks of estuary and sheltered bay <sup>27</sup>
<i>I. punctata</i>	Singapore, Malay Peninsula, Vietnam <sup>15</sup>	Forested mud flats/banks of estuary and sheltered bay <sup>27</sup>

There was some variation among crabs showing circular and vertical waves. The vertical type was further categorized into two subtypes, forward (*I. delsmanni* and *I. serrata*) and non-forward (*I. dentimerosa* and *I. pingi*), depending upon whether the chelipeds were extended forward while raised. The vertical wave in *I. dentimerosa* involved a short pause during the maximum elevation of the chelipeds, while three species (*I. delsmanni*, *I. serrata*, and *I. pingi*) lacked the pause.

Chela quivering in which a crab trembles one or both cheliped between consecutive waves was observed in 9 of 17 species of *Ilyoplax* (Table 2). In most cases, quivering was made for several bouts while positioned in front of the buccal region. In the two species that lack waving display, *I. obliqua* frequently exhibited chela quivering, while *I. punctata* did not. However, *I. punctata* was treated as a behavioral unknown species in the trait mapping because the observation (only one-day) was not enough to ensure absence of waving and chela quivering.

#### Trait Mapping

The tree estimated by the MP methods optimized for waving forms infers a single origin of the circular wave early in the history of the lineage (Fig. 4). Vertical waves may have evolved independently three times from the circular wave (once in the common ancestor of *I. serrata* and *I. delsmanni*, and once in *I. pingi* and in *I. dentimerosa*). The asymmetrical wave form appears to have evolved once from the common ancestor of *I. tansuiensis* and *I. orientalis*. The

superimposition of the chela quivering behavior on the inferred tree indicated that such behavior has a single origin (Fig. 5). Each of the two major clades inferred from the present phylogenetic analysis showed a specific character state of chela quivering. One clade included species with chela quivering and the other included those without chela quivering. The minor differences of the topology obtained from the three different inference methods (MP, NJ, NP analysis) did not affect reconstruction of the waving form and chela quivering evolution.

#### DISCUSSION

Present phylogenetic hypothesis suggests that the circular form is plesiomorphic in species of *Ilyoplax* and later changed to another form (vertical or asymmetrical) in some of the lineages and species. A field observation showed the wave motions of the vertical form were somewhat different among *I. dentimerosa*, *I. pingi*, and *I. delsmanni* and *I. serrata*. In *I. dentimerosa*, the chelipeds paused briefly at maximum elevation of chelipeds, but not in *I. pingi*, *I. serrata*, and *I. delsmanni*. The chelipeds are extended forward while raised in *I. delsmanni* and *I. serrata*, but not in *I. dentimerosa* and *I. pingi*. These differences support the hypothesis that the vertical wave independently evolved multiple times in the lineage.

What factors, then, are responsible for the evolution of another form of waving? The habitat conditions of circular species are almost the same as those of species showing

Table 4. Co-occurrence in the geographic area as well as the habitat for each species of *Ilyoplax*. A dash indicates that waving behavior is absent. C, V and A represent circular, vertical and asymmetrical wave forms, respectively. Co-occurrence with congeneric species sources based on Table 3 as well as follows: <sup>1</sup>Wada (unpublished data); <sup>2</sup>Kitaura and Wada (unpublished data).

Wave form	Species	Co-occurring congeneric species with their wave form	
Circular	<i>I. dentata</i>	None	
	<i>I. deschampsi</i>	<i>I. dentimerosa</i> (V), <i>I. pingi</i> (V) <sup>1</sup>	
	<i>I. formosensis</i>	None <sup>1</sup>	
	<i>I. gangetica</i>	<i>I. delsmanni</i> (V), <i>I. orientalis</i> (A), <i>I. obliqua</i> (—)	
	<i>I. ningpoensis</i>	<i>I. serrata</i> (V), <i>I. tansuiensis</i> (A) <sup>1</sup>	
	<i>I. pusilla</i>	None	
	<i>I. strigicarpa</i>	None	
	<i>Ilyoplax</i> sp.	None	
	Vertical	<i>I. delsmanni</i>	<i>I. gangetica</i> (C), <i>I. orientalis</i> (A), <i>I. obliqua</i> (—)
		<i>I. dentimerosa</i>	<i>I. deschampsi</i> (C), <i>I. pingi</i> (V)
<i>I. pingi</i>		<i>I. deschampsi</i> (C), <i>I. dentimerosa</i> (V)	
<i>I. serrata</i>		<i>I. ningpoensis</i> (C), <i>I. tansuiensis</i> (A)	
<i>I. orientalis</i>		<i>I. gangetica</i> (C), <i>I. delsmanni</i> (V), <i>I. obliqua</i> (—) <sup>2</sup>	
Asymmetrical	<i>I. tansuiensis</i>	<i>I. ningpoensis</i> (C), <i>I. serrata</i> (V)	
	<i>I. obliqua</i>	<i>I. gangetica</i> (C), <i>I. delsmanni</i> (V), <i>I. orientalis</i> (A), <i>I. punctata</i> (—) <sup>2</sup>	
Absent	<i>I. punctata</i>	<i>I. obliqua</i> (—)	

other wave forms. Most species of *Ilyoplax* occur mainly on the open mud or sandy mud flats in the mid to high intertidal zone, except for a few rock-dwelling and forested species (Table 3). Thus, the hypothesis that wave form is an adaptation to the signaling environment is unlikely. In contrast, the loss of a waving display may be related to the signaling environment. *Ilyoplax obliqua* and *I. punctata* usually occur at mud banks within a mangrove forest, where visual communication seems to be more difficult than on exposed open mud flats. Habitat conditions, such as flat and exposed substrate, have been hypothesized to play an important role in the evolution of waving display in semi-terrestrial grapsoids and ocypodoids (Salmon and Atsides, 1968; Kitaura et al., 2002). In the species of *Ilyoplax* currently studied, the species living on flat and exposed substrates actually perform a waving display, but the species occurring in a vertical (mud bank) habitat in mangrove forests may not perform waving.

Table 4 lists co-occurring species (defined as the species co-occurring in the same habitat, with wide overlap in geography) for each species. This information was based on literature and our unpublished data (Table 3). Co-occurring species appear to show different wave forms; the same waving form is never observed among co-occurring species (except for *I. dentimerosa* and *I. pingi*, but wave motions of these two species are somewhat different, as mentioned above). Furthermore, all the species without co-occurring species (Table 4) exhibit circular waves. These findings indicate that the co-occurrence of congeneric species may be an important factor in the evolution of other forms of waving. This hypothesis is supported by comparison between species with and without co-occurring species which shows that change from circular waving to another wave form (vertical/asymmetrical) occurred significantly

Table 5. Wave form change relative to co-occurrence with congeneric species. Fisher's exact probability test:  $p = 0.028$ .

	Wave form change	
	Circular → Circular	Circular → Asymmetrical or vertical
Species without co-occurring	5	0
Species with co-occurring	3	6

more often in the presence of other co-occurring species (Table 5).

In contrast to wave forms, chela quivering was suggested to have evolved only once in a common ancestor of one of the two major clade in the lineage of *Ilyoplax*. This suggests that the evolution of chela quivering may be subject to unidentified phylogenetic constraints. In *I. obliqua*, chela quivering was frequently observed, but wave motions as performed in other species of *Ilyoplax* was not observed. The function of chela quivering has so far not been examined in *I. obliqua* or in other species of *Ilyoplax*. The function of chela quivering and the relationships between waving and chela quivering are topics for future study, perhaps to explain why waving does not occur, but why chela quivering is present in *I. obliqua*.

#### ACKNOWLEDGEMENTS

We thank Drs. P. N. Hong and D. V. Nhuong (Vietnam), and Mr. Sophon Havanond (Thailand) for their support during our field research. We also thank anonymous reviewers for providing helpful comments. This work was partly supported by the Research Fellowship of the Japan Society for the Promotion of Science for Young Scientists to J. K., and by Grants-in-Aid for Overseas Scientific Survey from the Japan Ministry of Education, Science, Sports and Culture to K.W.

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RECEIVED: 28 October 2005.

ACCEPTED: 20 March 2006.