BEHAVIOURAL AND MOLECULAR EVIDENCE FOR THE SYSTEMATIC POSITION OF *MACROPHTHALMUS* (*HEMIPLAX*) *HIRTIPES* HOMBRON & JACQUINOT, 1846, WITH COMMENTS ON MACROPHTHALMINE SUBGENERA (DECAPODA, BRACHYURA, MACROPHTHALMIDAE)

BY

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ABSTRACT

The analysis of 16s rRNA data from the New Zealand sentinel crab, *Macrophthalmus* (*Hemiplax*) *hirtipes* (Jacquinot & Hombron, 1846) (Macrophthalminae: Macrophthalmidae) shows that it is the most basal species in the genus *Macrophthalmus*, and behavioural trait mapping suggests that ancestral behaviours included extended cheliped fighting. Other behaviour found in some sentinel crabs, such as allocleaning and waving displays, were absent in the ancestor and are regarded here as derived traits. It is proposed that the two subgenera *Hemiplax* and *Tasmanoplax* be elevated to full generic status. Three subgenera appear to be polyphyletic: *Macrophthalmus* (26 species), *Mareotis* (14 species) and *Paramareotis* (4 species), and to resolve this problem molecular data from the remaining species will need to be added into the analysis. The Australasian fauna contains the four most basal genera of the Macrophthalminae: *Tasmanoplax*, *Hemiplax*, *Australoplax*, and *Enigmaplax*. Our hypothesis is that these were early off-shoots from Tethyan ancestor(s) that colonized Australasia.

RÉSUMÉ

L'analyse des données d'ARNr 16S du crabe sentinelle venant de la néo-zélandais, *Macrophthalmus* (*Hemiplax*) *hirtipes* (Jacquinot et Hombron, 1846) (Macrophthalminae: Macrophthalmidae) montre que cette espèce est la plus basale du genre *Macrophthalmus*, et l'analyse des traits comportementaux suggère que le comportements de ses les ancêtres incluaient des combats avec les chélipédes déployés. D'autres comportements observés chez certains crabes sentinelles, tels que le nettoyage de conspécifiques ou l'agitation des chélipèdes, devaient être

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absents chez les formes ancestrales et sont considérés ici comme des traits dérivés. Les deux sous-genres *Hemiplax* et *Tasmanoplax* sont élevés au niveau de genre à part entière. Trois sous-genres semblent être polyphylétiques: *Macrophthalmus* (26 espèces). *Mareotis* (14 espèces) et *Paramareotis* (4 espèces). Cependant, pour résoudre ce problème, des données moléculaires relatives aux espèces non disponibles devront être ajoutées à l'analyse. La faune australasenne referme les quatre genres de Macrophthalminae les plus basaux: *Tasmanoplax*, *Hemiplax*, *Australoplax* et *Enigmaplax*. Notre hypothèse est que ce sont les descendants de formes téthyennes ancestrales ayant colonisé l'Australasie dans un passé géologique distant.

INTRODUCTION

The evolution of social behaviour in sentinel crabs was investigated by Kitaura et al. (2006) who mapped the behaviours onto a molecular phylogeny of the ocypodoid genus *Macrophthalmus* Desmarest, 1823. As currently accepted, the macrophthalmid genus *Macrophthalmus* contains 55 species divided into eight subgenera (Ng et al., 2008). Recently two new species have been described (*M.* (*Macrophthalmus*) *ryukyuanus* Naruse & Kosuge, 2008; *M.* (*Mareotis*) *pistrosinus* Barnes & Davie, 2008) to make a total of 57 species. Mendoza & Ng (2007) discussed some of the complex aspects of these subgenera and provided a key to identify them (see also Komai et al., 1995; Nagai et al., 2006; Naruse & Kosuge, 2008; Davie, 2009; Barnes, 2010). In their study, Kitaura et al. (2006) included 21 species, representing six subgenera from Japan, Vietnam and Australia. The subgenera included are *Macrophthalmus*, *Mareotis*, *Chaenostoma* (as *Mopsocarcinus*), *Tasmanoplax*, *Paramareotis* and *Venitus*. However, they did not include the mono-specific subgenus *Hemiplax* Heller, 1865, which contains *Macrophthalmus* (*Hemiplax*) *hirtipes* (Hombron & Jacquinot, 1846) from New Zealand. The aim of this paper is to expand the analysis of the genus to include *Hemiplax* by introducing the New Zealand species into the molecular phylogeny, and comparing its social behaviour with other species of *Macrophthalmus*. The only subgenus not included is *Euplax* H. Milne Edwards, 1852, represented in the West Pacific by *Macrophthalmus* (*Euplax*) *dagohoyi* Mendoza & Ng, 2007. The inclusion of *Hemiplax* could potentially alter the conclusions of Kitaura et al. (2006) on the evolution of allocleaning, fighting, and cheliped waving displays. Finally, we present a partial reappraisal of the subgenera of *Macrophthalmus*, and propose that *Hemiplax* Heller, 1865, and *Tasmanoplax* Barnes, 1967, be elevated to full generic rank. Further revision will require more samples of 16s rRNA from the other subgenera and species of sentinel crabs.

MATERIALS AND METHODS

The species used to reconstruct the phylogeny of *Macrophthalmus* are listed in table I. Most of the sequences were from previous studies carried out by Kitaura et al. (1998, 2002, 2006). The new samples included for this study are *Macrophthalmus crinitus* (Kema, Indonesia, 4/Aug/2001), *M. hirtipes* (Christchurch, NZ, 17/March/2007) and *M. philippinensis* (Iriomote Is., Japan, 10/Aug/1999) making a total of 22 species. Total genomic DNA was extracted from muscle tissue of walking legs using QuickGene DNA tissue kit (FUJIFILM). Selective amplification of portions of the mitochondrial large ribosomal subunits (16s rRNA) were performed as reported by Kitaura et al. (2002, 2006), using a combination of the primers L1496i, L2510C, H2492i, H2716i, and H3062. The PCR products were purified using ExoSAP-IT (USB Corporation) and then sequenced with the ABI BigDye terminator mix in an ABI Prism 310 Genetic Analyzer. All final sequences were obtained from both strands for verification.

The sequences were initially aligned using CLUSTAL W (Thompson et al., 1994) with default gap penalties, subsequently being modified slightly by eye. Positions that could not be aligned accurately were excluded from the data sets. Phylogenetic relationships were analyzed by the maximum likelihood (ML) method, using the software package PAUP* ver. 4.0b8 (Swofford, 1998), and Bayesian analysis (BAY) as implemented in MrBayes v. 3.1.2 (Huelsenbeck and Ronquist, 2001). Sequences were first analyzed with the software Mrmodeltest 2.2 (Nylander, 2004) in order to find the evolutionary model that best fits the data. The ML analysis was performed using a heuristic search algorithm. Search options included obtaining the starting tree by step-wise additions, using the random sequence addition, and tree bisection, and reconnection branch swapping. To assess statistically the consistency of nodes, bootstrap analyses as a heuristic search were applied with 100 replicates. The BAY analysis was performed by running a Markov chain Montecarlo (MCMC) algorithm for 2 000 000 generations, sampling one tree every 100 generations. The first 10% of generations were discarded (burn-in) for the tree building analysis. The posterior probabilities of the phylogeny were determined by constructing a 50% majority-rule consensus of the remaining trees.

In-situ observations on the social behaviour of *Macrophthalmus hirtipes* were carried out during day-time low tides at the Avon-Heathcote Estuary, Christchurch, New Zealand. Ex-situ observations on captive male and female crabs from the same area were carried out in a 32 × 32 × 10 cm plastic tank with its bottom covered by mud-sand sediment from the their natural habitat.

TABLE I

List of *Macrophthalmus* Desmarest, 1823, species used for DNA analysis. Arrangement of species into subgenera follows that used by Ng et al. (2008)

Species	Collection locality	Genetic database accession no.
Subgenus *Chaenostoma* Stimpson, 1858		
M. boscii Audouin, 1826	Minamihaemida, Iriomote Is., Okinawa, Japan	AB101492
Subgenus *Hemiplax* Heller, 1865		
M. hirtipes Hombron & Jacquinot, 1846	Avon-Heathcote Estuary, Christchurch, New Zealand	AB440189
Subgenus *Macrophthalmus* Desmarest, 1823		
M. abbreviatus Manning & Holthuis, 1981	Isahaya Bay, Nagasaki, Japan	AB101482
M. brevis (Herbst, 1804)	Sashiki, Okinawa, Japan	AB058631
M. banzai Wada & K. Sakai, 1989	Yokoura, Shirahama, Japan	AB002132
M. convexus Stimpson, 1858	Iriomote Is., Okinawa, Japan	AB101483
M. darwinensis Barnes, 1971	Ludmilla Creek, Darwin, Australia	AB101486
M. milloti Crosnier, 1965	Ishigaki Is., Okinawa, Japan	AB101484
M. philippinensis Serène, 1971	Iriomote Is., Okinawa, Japan	AB537377
M. sulcatus H. Milne Edwards, 1852	Can Gio, Vietnam	AB101485
Subgenus *Mareotis* Barnes, 1967		
M. crinitus Rathbun, 1913	Kema, Indonesia	AB537376
M. definitus Adams & White, 1849	Urauchi River, Iriomote Is., Okinawa, Japan	AB101487
M. japonicus (De Haan, 1835)	Tanabe Bay, Shirahama, Wakayama, Japan	AB101488
M. pacificus Dana, 1851	Ludmilla Creek, Darwin, Australia	AB101490
M. pistrosinus Barnes & Davie, 2008	Small Boat Harbour, Carnarvon, Australia	AB101489
M. tomentosus Eydoux & Souleyet, 1842	Giao Thuy, Vietnam	AB101491
Subgenus *Paramareotis* Komai, Goshima & Murai, 1995		
M. boteltobagoe Sakai, 1939	Horikawa, Okinawa, Japan	AB101494
M. erato De Man, 1888	Ludmilla Creek, Darwin, Australia	AB101496
M. holthuisi Serène, 1973	Iriomote Is., Okinawa, Japan	AB101495
M. quadratus A. Milne Edwards, 1873	Sashiki, Okinawa, Japan	AB058633

TABLE I
(Continued.)

Species	Collection locality	Genetic database accession no.
Subgenus *Tasmanoplax* Barnes, 1967		
M. latifrons Haswell, 1882	Laverton Creek, Melbourne, Australia	AB101493
Subgenus *Venitus* Barnes, 1967		
M. latreillei (Desmarest, 1822)	Ludmilla Creek, Darwin, Australia	AB058632

Half of the tank was filled with 8 cm of sandy-mud with artificial burrows on the exposed side, while the other half was covered with a 1 cm layer of sediment and 1 cm of seawater. Kitaura et al. (2006) analyzed *Macrophthalmus* behaviour in terms of 1) allocleaning wherein a crab forages on the body of another conspecific individual; 2) fighting wherein two kinds were recognized in males (a — grasping type where the chelipeds are used to grasp the body of an opponent, followed by pushing; b — claw-extension type where grasping is followed by cheliped lateral extension and touching, face to face) and in females (c — leg-intertwining fighting; d — leg-extending fighting, see Kitaura & Wada, 2004); 3) waving displays in which four types were recognized (involving different vertical and lateral components) and mating. Both allocleaning and waving are components of reproductive behaviour.

RESULTS

Molecular phylogeny

A total of 977 bp (including gaps) were used for phylogenetic analysis. The selected model of DNA substitution by hLRT was the GTR + I + G model. Parameter values under the selected substitution model are as follows: base frequencies freqA = 0.4113, freqC = 0.0469, freqG = 0.1357, freqT = 0.4061; nucleotide substitution rates A↔C 0.6129, A↔G 7.5425, A↔T 0.7671, C↔G 0.5392, C↔T 16.3294, G↔T 1.0000; proportion of invariable sites (I) = 0.33; variable sites followed a gamma distribution with shape parameter = 0.56.

The maximum likelihood and Bayesian topology trees for 22 species of *Macrophthalmus* (see table I) are shown in figs 1 & 2. Two Australasian species, *M. hirtipes* and *M. latifrons* emerge as basal species along with two

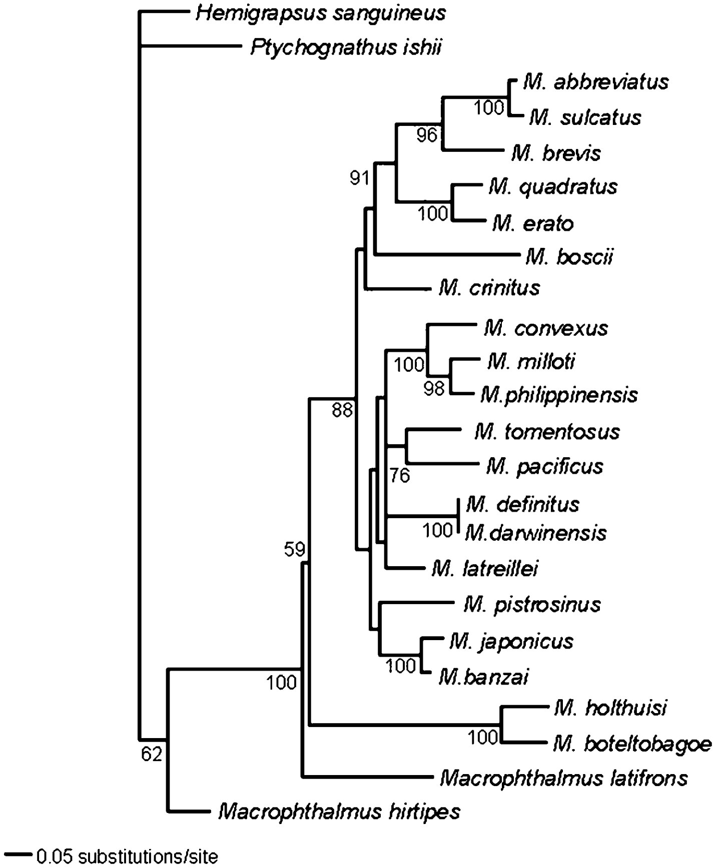

Fig. 1. Maximum likelihood tree for *Macrophthalmus* for 22 species and outgroup taxa using GTR + I + G model of sequence evolution. Clade supported by BP $\geqslant$ 50% are indicated under the branches.

Japanese species, *M. holthuisi* and *M. boteltobagoe*. The sister group to these four species includes the remaining 18 species which form two lineages, one with seven and the other with 11 species. *Macrophthalmus holthuisi* and *M. boteltobagoe* have been placed by Ng et al. (2008) in the subgenus *Paramareotis*. However, the other two species in this subgenus, *M. erato* and *M. quadratus*, remain together but come out nested in the smaller lineage (above). On the basis of our data, five groups are discernible: 1) *M. hirtipes* group; 2) *M. latifrons* group; 3) *M. holthuisi* + *M. boteltobagoe*; 4) *M. crinitus* + *M. boscii* + *M. brevis* + *M. abbreviatus* + *M. sulcatus* + *M. quadratus* + *M. erato* group; and 5) *M. convexus* + *M. milloti* + *M. philippinensis* + *M. pistrosinus* + *M. japonicus* + *M. banzai* + *M. tomentosus* + *M. pacificus* + *M. latreillei* + *M. definitus* + *M. darwinensis* group. The subgenus *Chaenostoma*,

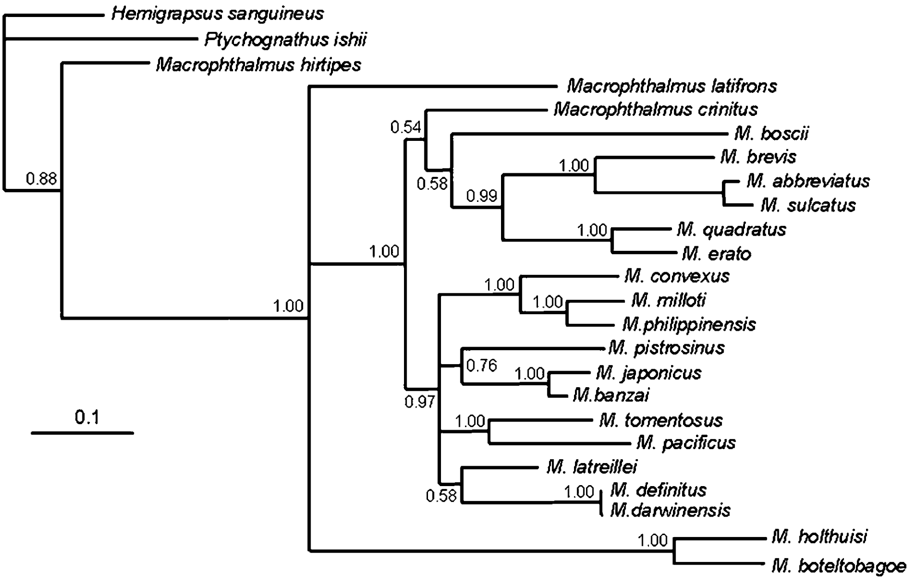

Fig. 2. Bayesian topology tree for 22 species of *Macrophthalmus*. Posterior probabilities are shown around branches.

represented by *M. boscii*, is nested within the fourth lineage and the subgenus *Venitus*, represented by *M. latreillei*, is nested within the fifth lineage (above). The species representing the subgenera *Macrophthalmus* and *Mareotis* do not group together and are scattered across both the fourth and fifth lineages (above). The Australasian fauna contains representatives of four of the five lineages, including the two most basal, while the Japanese fauna contains representatives of three nested lineages and thus is more derived.

Behaviour

Rather than repeat the field observations made by Beer (1959), we summarize his results here, and provide our own additional observations that allow full comparison of *Macrophthalmus hirtipes* with those made on the other species by Kitaura et al. (2006). Neither allocleaning nor waving behaviours have been recorded in *M. hirtipes* (C.L. McLay, unpublished). The only social behaviour that has been recorded in this species is fighting (see Beer, 1959). When two crabs meet in the vicinity of a burrow, the owner adopts a threat posture in which the chelipeds are raised off the sand with opened fingers and the all other pereopods extended (fig. 3A). If the aggressor adopts the same posture, then the crabs may draw together with chelipeds spread, revealing the bright red-white patterned inner surface of the chelipeds. It should be noted,

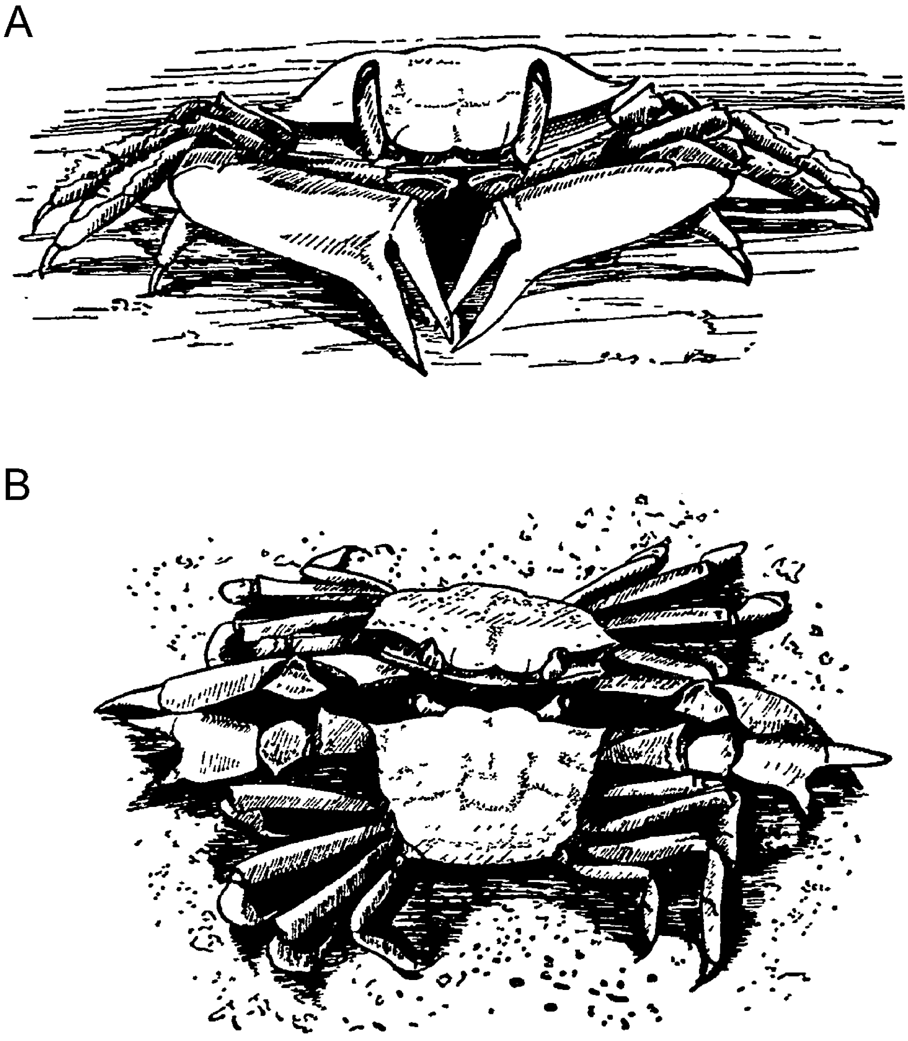

Fig. 3. *Macrophthalmus* (*Hemiplax*) *hirtipes*. A, threat display; B, cheliped-extension Type 2b fighting face to face (after Beer, 1959, fig. 1).

however, that the strong red-white pattern is not seen in males from all localities. The pair may face each other for up to five minutes (fig. 3B). One contest lasted 70 minutes with several threats and pursuits, but the same crab was dominant. Full cheliped-extended fighting was only observed between *M. hirtipes* males, and seemed to be a ritualized behaviour that did not result in limb loss. Real fights, which might result in injury, are reduced to a minimum. Often a crab that had vigorously and successfully defended his burrow would walk off leaving the burrow to other males, apparently never to return (Beer, 1959). The fighting behaviour of *M. hirtipes* males seems to involve a minimum grasping and grappling with opponents (type 2a) and usually involves ritualized cheliped extension (type 2b of Kitaura et al., 2006). Beer (1959) observed aggressive males evicting each other from a burrow showing behaviour that could best be described as "mischievous"! Female *M. hirtipes* do not show the leg-intertwining fighting and leg-extending fighting that are seen in some *Macrophthalmus* species by Kitaura & Wada (2004).

DISCUSSION

Most species of *Macrophthalmus* show some form of courtship waving display, and fall into one of two clades depending on features of the display (see Kitaura et al., 2006, fig 6). However, from their analysis, the presence/absence of waving in the ancestor was equivocal. *M.* (*Tasmanoplax*) *latifrons* was the most basal of 19 species in the genus, and it has a well developed vertical forward-pointing wave, but the two Japanese species, *M.* (*Paramareotis*) *holthuisi* and *M.* (*Paramareotis*) *boteltobagoe* that are nested within the sister group to *M.* (*Tasmanoplax*) *latifrons*, both lack a waving display. The rest of the sister group has lateral waving. The new data presented here shows that *M. hirtipes* is the sister species to the entire group studied by Kitaura et al. (2006) and it lacks any kind of waving display. This supports the hypothesis that the ancestor of *Macrophthalmus* lacked any courtship display, and that waving has evolved independently in *M.* (*Tasmanoplax*) *latifrons* and in the lateral waving group. The most likely explanation of the absence of waving in the two Japanese species is that they have retained the ancestral condition. Within the lateral waving group, *M. abbreviatus* seems to have lost the display secondarily because it is derived from ancestors that waved (Kitaura & Wada, 2004). Looking at reproductive behaviour, only *M. japonicus* and *M. banzai* are known to mate in burrows, while all the others mate on the surface (Kitaura et al., 2006). Both of these species are well nested within the molecular phylogeny, suggesting that this kind of mating is derived and, by implication, that surface mating is ancestral. Jennings et al. (2000) only found surface mating without mate guarding in *M. hirtipes*.

The fighting behaviour in *Macrophthalmus* almost exclusively involves male crabs (Griffin, 1968), and it can be categorized as grasping or claw-extended fighting. Kitaura et al. (2006) concluded that grasping fighting evolved from claw-extended fighting which characterized the basal species *M. latifrons* (and *M. holthuisi* + *M. boteltobagoe*). *Macrophthalmus hirtipes* has the same claw-extended fighting behaviour seen in *M. latifrons* (see Griffin, 1968), reinforcing their conclusion. However, this requires us to postulate further that claw-extended fighting evolved secondarily in four species of the subgenus *Macrophthalmus* which are part of the sister group (fig. 1).

There is an important link between behaviour and morphology in sentinel crabs. Length of eyestalks ranges from relatively short in *Macrophthalmus* (*Hemiplax) hirtipes* (ratio of sum of both eyestalk lengths to CW = 0.65) to relatively long in *Macrophthalmus* (*M.*) *serenei* Takeda & Komai, 1991 (ratio = 1.5) where the eyes are too long to be accommodated by the orbits. Barnes (1976) recognized the "*M. telescopicus* Owen, 1839" group of eight species within the subgenus *Macrophthalmus* (including *M.* (*M.*) *serenei*). In order to accommodate long eyestalks in these species the distance between them has narrowed and the frontal width of the carapace has increased. Thus we can recognize two groups within *Macrophthalmus*: narrow-fronts and broad-fronts. In our analysis, we regard the broad-front + short eyestalks character as plesiomorphic and narrow-front + long eyestalks as derived. We hypothesize that long eyestalks evolved as predation risk increased and that this resulted in the range of variations of frontal morphology seen within present day *Macrophthalmus*. Our molecular phylogenetic analysis supports this hypothesis that broad-fronts are basal and narrow-fronts are derived: for e.g. *Macrophthalmus* (*H.*) *hirtipes* is basal while *M.* (*M.*) *milloti* is derived.

SYSTEMATICS

Superfamily OCYPODOIDEA Rafinesque, 1815

Family MACROPHTHALMIDAE Dana, 1852

Subfamily MACROPHTHALMINAE Dana, 1852

Hemiplax Heller, 1865 status nov.

Diagnosis. — Carapace quadrangular, elongated, carapace length ca. 0.7 times carapace width; regions well defined, flattened, finely granulate; with transverse, oblique granular rows on branchial region Frontal margin ca. 0.3 times carapace width, protruding downward ventrally. Epigastric cristae not distinct, gastric cristae more distinct. Anterolateral margin of carapace with three teeth including post-orbital tooth; posterolateral margin convex, narrowing towards posterior border. Orbits large, elongated, without infra-orbital crest. Eyestalk thick, short ca. 0.25 times carapace width. Antennules transverse or slightly oblique; inter-antennular septum narrow. Third maxilliped narrow, with a small gape when closed; merus subequal to ischium; merus, ischium sculptured. Epistome broad, posterior margin slightly sinuous, almost straight. Chelipeds equal, longitudinal carina present partially on lower margin, partially on outer surface of adult male chela; no tubercle on inner surface of palm; carpus with spine on upper, inner margins; stout dactylus and immovable finger elongate, deflexed, without differentiation, inner surfaces densely setose. Pereopods slender, margins glabrous; dactyli long, curved, unarmed; merus with subdistal tooth on anterior margin. Sixth abdominal male somite broad, margins convex (modified from Barnes, 1967: 204).

Hemiplax hirtipes (Hombron & Jacquinot, 1846) comb. nov.
(fig. 4A-C)

Cleistostoma? *hirtipes* Hombron & Jacquinot, 1846: 69, pl. 6, fig. 3C.
Metaplax hirtipes Heller, 1862: 521.
Hemiplax hirtipes — Heller, 1865: 40, pl. 4 fig. 3; Miers, 1876: 34; Filhol, 1885: 365; Miers, 1886: 251; Chilton, 1909: 608; Tesch, 1918: 57; Kemp, 1919: 384; Oliver, 1923: 540; Chilton & Bennett, 1929: 759; Richardson, 1949: 36; Ralph & Yaldwyn, 1956: 74, fig. 41; Bennett, 1964: 85; Dell, 1968: 26; Wear, 1968: 698, figs. 1-9 (zoea); Rice, 1980: 342.
Macrophthalmus hirtipes — Thompson, 1902, 462; 1913, 237; Wear & Fielder, 1985: 76, figs. 197-200; McLay, 1988: 336, fig. 71.
Macrophthalmus (*Hemiplax*) *hirtipes* — Barnes, 1967: 236, pl. 3d, fig. 12; 1970: 242; Ng et al., 2008: 237 (list).

Type and only included species. — *Macrophthalmus* (*Hemiplax*) *hirtipes* (Hombron & Jacquinot, 1846).

The type specimen of *Macrophthalmus* (*Hemiplax*) *hirtipes* (a dried male, 29.9 × 17.6 mm, MNHN-B4622) was collected when the "Pole Sud Expedition" vessels "Astrolabe" and "Zélée" visited New Zealand between March-April, 1840. Hombron & Jacquinot (1846: 69) stated that the type specimen was collected in Samoa (1838), but this seems to be an error because their illustration of "*Cleistostoma*? *hirtipes*" shows a crab identical to the New Zealand *Hemiplax hirtipes* (see McLay, 1988: 336). Furthermore, no additional specimen has ever been found in Samoa since 1840, and this species is very common in New Zealand estuaries and on sheltered muddy shores. Before visiting Samoa, the expedition had spent some time in New Zealand waters so it is likely that the type specimen was mislabelled at some stage. Precisely where the type specimen came from is not recorded, but given the time that the expedition spent in each location, the most likely places are Otago Harbour, Akaroa Harbour (South Island) or the Bay of Islands (North Island) of New Zealand.

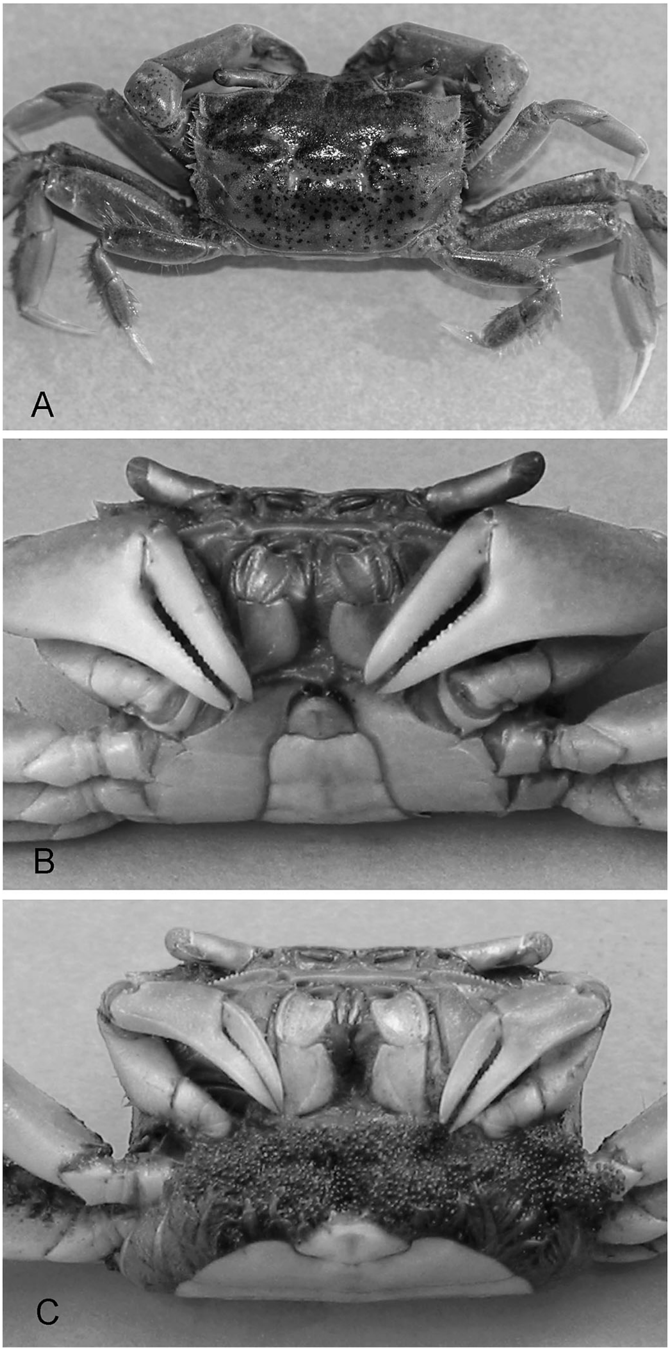

Fig. 4. *Hemiplax hirtipes*. A, dorsal view, male 22.0 × 15.6 mm; B, frontal view, male 22.0 × 15.6 mm; C, frontal view, female 17.0 × 11.7 mm (personal collection, CLM).

The holotype of *Metaplax hirtipes* Heller, 1865, has been reduced to a few fragments (see Pretzmann, 1974, fig. 15), but the original description and figure (Heller, 1865: 40, pl. 4, fig. 3) leave no doubt as to the identity of the specimen. It was collected by scientists aboard the Austrian frigate "Novara" sometime between December 22, 1858 and January 8, 1859, from the vicinity of Auckland, New Zealand (Heller, 1865: 41).

The authorship of the species name requires some clarification and explanation. Clark & Crosnier (2000) and Ng et al. (2008) give the name as *Macrophthalmus* (*Hemiplax*) *hirtipes* (Jacquinot, in Hombron & Jacquinot, 1846) although Holthuis (2002: 421) has advised that the name should not be attributed to just Jacquinot. We here follow the opinion of Holthuis (2002).

Hemiplax hirtipes is a taxon endemic to New Zealand, occurring on the North, South and Stewart Islands as well as Campbell Is (52°32′S, 169°10′E) south of New Zealand (McLay, 1988: 336). Barnes (1967) had included *Macrophthalmus* (*Hemiplax*) *hirtipes* and *M. boteltobagoe* Sakai, 1939, in *Hemiplax*, but the latter species does not belong in this genus based on the adult, DNA and behavioral data (see above). *M. boteltobagoe* was later transferred to the subgenus *Paramareotis* by Komai et al. (1995).

Tasmanoplax Barnes, 1967, status nov.

Diagnosis. — Carapace quadrangular, broad, carapace length ca. 0.6 times carapace width; regions well defined, flattened, finely granulate with two longitudinal granular rows in anterior/posterior position on branchial region; without granules. Frontal margin ca. 0.2 times carapace width. Epigastric cristae not distinct, gastric cristae more distinct. Anterolateral margin of carapace with three teeth including post-orbital tooth, anterolateral teeth with base broad, rectangular in shape (chisel-like); posterolateral margin subparallel. Orbits large, elongated, cornea extends to base of external orbital angles. Ocular peduncles long, narrow, ca. 0.25 times carapace width. Antennules transverse or slightly oblique; inter-antennular septum narrow. Third maxilliped narrow, close with a small gape; merus subequal to ischium; merus, ischium sculptured. Epistome broad, posterior margin very convex. Chelipeds equal, longitudinal ridge present on outer surface of male chela; without tubercle on inner surface of palm; carpus without spine; dactylus and immovable finger elongate, deflexed, without differentiated tooth. Pereopods slender, margins glabrous; dactylus long, curved, unarmed; merus with subdistal tooth on anterior margin. Sixth abdominal male somite narrowing on posterior margin (modified from Barnes, 1967: 204).

Macrophthalmus latifrons Haswell, 1882, comb. nov.
(fig. 5)

Macrophthalmus latifrons Haswell, 1882, 549: 1882, 90; Tesch, 1915: 189.
Microphthalmus (sic) *latifrons* — Fulton & Grant, 1906: 19.
Hemiplax latifrons — Etheridge & McCulloch, 1916: 13, pls. 4, 6; Hale, 1927: 186, fig. 187; Tweedie, 1941: 25, fig. 10; Griffin, 1968: 291.
Macrophthalmus (*Tasmanoplax*) *latifrons* — Barnes, 1967: 239, pl. 4a, fig. 13; Ng, et al., 2008: 238 (list).

Type and only species. — *Macrophthalmus latifrons* Haswell, 1882; gender feminine. By original designation.

Remarks. — The genera, *Tasmanoplax* and *Hemiplax*, can be easily distinguished by the relative width of the frontal margin (relatively narrow in *Tasmanoplax* versus broader in *Hemiplax*), the shape of the epistome (central protuberance present in *Tasmanoplax* versus straight in *Hemiplax*) and shape of sixth abdominal segment (margins narrowing posteriorly in *Tasmanoplax* versus convex in *Hemiplax*) (cf. Barnes, 1967).

Tasmanoplax latifrons is a monotypic genus endemic to Australia and is found on intertidal mudflats and sea grass beds in Victoria, South Australia and Tasmania (Poore, 2004), a similar habitat to that of *H. hirtipes* from New Zealand.

Fig. 5. *Tasmanoplax latifrons*. Male 21.4 × 12.8 mm, Trial Bay, Tasmania (registration number J17036, Museum of Victoria), dorsal view.

DISCUSSION

The genus *Macrophthalmus* currently contains 57 species, and we have sequenced 39% (22 species) of the species for our reconstruction of the phylogenetic relationships. Representatives of seven of the eight subgenera are included, and only *Euplax* H. Milne Edwards, 1852 is missing (see Mendoza & Ng, 2007, who discuss the history of this subgenus). All of the species in *Hemiplax*, *Paramareotis* and *Tasmanoplax* are included in the analysis. It is clear from these data sets that the subgenera *Paramareotis*, *Mareotis* and *Macrophthalmus* are polyphyletic. *Macrophthalmus* (*Paramareotis*) *quadratus* (type species) and *M.* (*P.*) *erato* are sister species, grouped with three species of *Macrophthalmus*, while *M.* (*P.*) *holthuisi* and *M.* (*P.*) *boteltobagoe* are together sister species of all the other species sequenced, except for *Hemiplax* and *Tasmanoplax*. Similarly, the subgenera *Mareotis* and *Macrophthalmus* are polyphyletic. Our analysis identifies five possible lineages designated 1)-5) above (see Results above). Two lineages are represented by single species from two subgenera, *Hemiplax* and *Tasmanoplax*, both from Australasia, and are clearly separated from the others. As such, we have proposed that these two subgenera be raised to full generic status. Ng et al. (2008: 238) noted the unsatisfactory nature of the division of the genus *Macropthalmus* into subgenera and suggested that *Euplax* H. Milne Edwards, 1852, and *Venitus* Barnes, 1967, could be treated as good genera. Unfortunately we were only able to include one species belonging to *Venitus*, *M.* (*Venitus*) *latreillei*, so we cannot comment on the likely position of the other six species within the molecular phylogeny; but for the moment we accept the generic status of *Venitus* as suggested by Ng et al. (2008). Later, Davie (2009) proposed that two broad-fronted species (*Macrophthalmus* (*Chaenostoma*) *boscii* Audouin, 1826 (type species of the subgenus), and *Macrophthalmus* (*Chaenostoma*) *punctulatus* Miers, 1884, should be included in *Chaenostoma* Stimpson, 1858, and it be elevated to generic status. The other subgenera in our analysis are multi-specific, and their composition could well change when the remaining species are sequenced and added to the mix. The question of whether the "*M. telescopicus*" group (see Naruse & Kosuge, 2008) is monophyletic is unresolved: it is possible that this is the case because the two species from this group that we have included in our phylogeny (*M.* (*M.*) *milloti* and *M.* (*M.*) *philippensis*) are derived sister species, but the other species will need to be added to the analysis. As it stands now, 45 species remain in the genus *Macrophthalmus*, but further revision is clearly necessary.

The case for elevation of *Hemiplax* is further supported by differences in larval structure. The first stage *Macrophthalmus* zoea are known from seven subgenera (12 species), including *H. hirtipes* by Wear (1968). The first zoea of *H. hirtipes* differs from *Macrophthalmus* zoea in the following characters: presence of large lateral carapace spines, absence of dorso-lateral processes on abdominal somite 3, well developed antennal exopod with two medial setae, and long carapace and telson spines (Fielder & Greenwood, 1985, 1986). Spination of the first zoea of *Chaenostoma punctulatus* deviates from the typical *Macrophthalmus* pattern of rostral + dorsal spines, by only having a short rostral spine and differs from *H. hirtipes* in lacking lateral spines (Fielder & Greenwood, 1986). The larvae of *Tasmanoplax* are not known.

Barnes' (1967) evolutionary hypothesis on the origins of the genus *Macrophthalmus* envisaged the ancestor with the following features: smooth or moderately granular subquadrate carapace, with a broad front; short ocular peduncles; semi-globose male chelae, with moderately long straight fingers, the immovable finger being straight; slender pereopods; merus and ischium of third maxillipeds subequal; epistome straight; first two somites of male abdomen small, the other somites with straight lateral margins evenly convergent from the third to the sixth somite; and slightly curved or straight first male gonopod. This ancestral form of *Macrophthalmus* was probably found under stones or occupied small burrow microhabitats in muddy sand, littoral or estuarine environments. It probably relied, on its small size and reclusive habits to escape from predation (Barnes, 1967). Barnes (1967) believed that predation was the dominant selective force influencing sentinel crab evolution, and he considered that the extant species closest to the ancestral state was *Macrophthalmus boscii* Audouin, 1826 (in the subgenus *Mopsocarcinus*). *Mopsocarcinus* is no longer considered valid and the species that Barnes included are now placed under *Chaenostoma* and *Paramareotis* (see Ng et al., 2008: 237). With *Mopsocarcinus* as ancestral, he identified five independently derived lineages: *Mareotis*, *Macrophthalmus*, *Venitus*, *Tasmanoplax* and *Hemiplax*. Based the distribution of species known at that time, Barnes (1967: 250) hypothesized that the genus *Macrophthalmus* arose in the Southeast Asian region.

Clearly the evidence presented herein supports a totally different picture of phylogenetic relationships of the species of *Macrophthalmus* from that envisaged by Barnes (1967). The species closest to the ancestral condition for the Macrophthalminae are *H. hirtipes* and *T. latifrons*, which could have been early off-shoots from a Tethyan ancestor that colonized Australasia. Three Australian monospecific genera, *Australoplax* Barnes, 1966, *Enigmaplax* Davie,

TABLE II
Summary of the numbers of genera and species of sentinel crabs in the Macrophthalminae

Genus	Number of species
Australoplax Barnes, 1966	1
Chaenostoma Stimpson, 1858	3
Enigmaplax Davie, 1993	1
Euplax H. Milne Edwards, 1852	2
Hemiplax Heller, 1865	1
Lutogemma Davie, 2009	1
Macrophthalmus Desmarest, 1823	44
M. (Macrophthalmus) Desmarest, 1823	26
M. (*Mareotis*) Barnes, 1967	14
M. (*Paramareotis*) Komai, Goshima & Murai, 1995	4
Tasmanoplax Barnes, 1967	1
Venitus Barnes, 1967	7
Total Number of Species	61

1993, and *Lutogemma* Davie, 2009, along with *Chaenostoma* Stimpson, 1858 and *Macrophthalmus* Desmarest, 1823, make up the subfamily Macrophthalminae. The exact phylogenetic relationships of all these genera remain to be established, in particular whether they represent one or several colonisations. Davie (2009) suggested that the broad-fronted species belonging to *Mareotis*, *Chaenostoma*, *Hemiplax*, *Tasmanoplax* and *Australoplax* might form a separate lineage from the narrow-fronted species that include *Lutogemma*. We summarize the present state of the genera and species in the Macrophthalminae taking into account the present results and the recent arguments presented by other authors (table II).

ACKNOWLEDGEMENTS

We would like to thank Peter Ng, Peter Davie and Shane Ahyong for valuable discussions. We are grateful to Gary Poore, Museum Victoria, Melbourne, who kindly provided the photographs of *Tasmanoplax latifrons*. This work was supported by Grants-in-Aid for Scientific Research from Japan Society for Promotion of Science to KW.

It is a pleasure and a privilege for us to acknowledge the contribution that Lipke Holthuis made to the study of decapod Crustacea. Just when you thought you “knew it all”, and asked Lipke a question, hoping it might lead to an opportunity to show him how much you knew about your animal, you would

usually find yourself on a new learning curve as he reminded you about a detail that you had overlooked. The authorship of the species under consideration here, *Hemiplax hirtipes* is a case in point (see above). What secrets there were on his bookshelf and in his own memory! We can only hope that we managed to ferret most of them out before he departed.

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First received 4 December 2009.
Final version accepted 25 March 2010.