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Reconstruction of phylogenetic relationships within Grapsidae (Crustacea: Brachyura) and comparison of trans-isthmian versus amphi-atlantic gene flow based on mtDNA

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Abstract

Following taxonomic revisions in recent years, the originally large family Grapsidae MacLeay, 1838 has become a relatively small and morphologically homogeneous family in terms of adult and larval morphology. Most available molecular studies including more than one genus of the family have also suggested monophyly of the corresponding taxa. However, no single phylogenetic study has ever included all constituent genera of the Grapsidae. In the current study, a molecular phylogeny based on sequences of the mitochondrial 16S rRNA gene from all eight grapsid genera and 34 species is presented and suggests that up to four genera are not monophyletic. This is mainly due to the polyphyletic nature of the genus *Pachygrapsus* which can be found in six different lineages of the phylogeny, suggesting that the genus currently does not represent a single evolutionary lineage and is in need of taxonomic revision. Amphi-atlantic and trans-isthmian species pairs or populations in four genera are compared and reveal relatively constant and pronounced divergences across the Panama Isthmus as opposed to moderate divergences across the Atlantic Ocean, thereby suggesting occurrence of gene flow across the Atlantic Ocean during the past three million years.

Keywords: Molecular phylogeny; Taxonomy; Thoracotremata; 16S rRNA; mtDNA; Transisthmian

1. Introduction

For a long time, the brachyuran family Grapsidae was a large assemblage of thoracotreme (see Guinot, 1978) crabs including representatives of more than 50 genera that are now allocated into their own families, i.e. Glyptograpsidae Schubart, Cuesta and Felder, 2002, Plagusiidae Dana, 1851, Percnidae Števčić, 2005 (see Schubart and Cuesta, 2010), Sesarmidae Dana, 1851, Varunidae H. Milne Edwards, 1853, Xenograpsidae Ng, Davie, Schubart and Ng, 2007, and Grapsidae MacLeay, 1838 *sensu stricto* (s. str.). After comparing morphological and molecular evidence, von Sternberg and Cumberlidge (2000) and Schubart et al. (2000) independently suggested to elevate all grapsid subfamilies to family level. This appeared necessary in order to place these taxa at the same taxonomic level as the closely related family Gecarcinidae MacLeay, 1838. Consequently, the Grapsidae s. str. became a relatively small (40 species) and morphologically homogeneous family in terms of adult (Schubart et al., 2002) and larval (Cuesta and Schubart, 1999; Cuesta et al., 2011) morphology. A number of molecular phylogenies which included more than one genus of the family (Schubart et al., 2000, 2002, 2006a; Kitaura et al., 2002; Ng et al., 2007; Schubart and Cuesta, 2010) suggested

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monophyly of the corresponding taxa, with the exception of the study by Kitaura et al. (2002), which showed a basal position of *Grapsus albolineatus* compared to *Metopograpsus thukuhar*, *Pachygrapsus minutus* and other Thoracotremata. However, all these studies had a limited taxon coverage, the most complete being the ones by Schubart et al. (2000) and Ng et al. (2007), which both included single representatives of six different genera. Therefore, the aim of this study is to construct a molecular phylogeny including all eight constituent genera of the family Grapsidae with multiple species representation per genus to reconstruct inter- as well as intrageneric evolutionary relationships.

Four genera of Grapsidae have representatives on both Atlantic coastlines (West Africa and Eastern America) and on the Eastern Pacific coast (Western America), which have been recognized and accepted as sister taxa, i.e. *Geograpsus*, *Goniopsis*, *Grapsus* and *Pachygrapsus*. In some cases these allopatric lineages are considered species, like in *Goniopsis*, whereas in other cases these lineages are considered intraspecific populations as in the case of *Geograpsus lividus* and until recently *Pachygrapsus transversus* (before Schubart et al., 2006b revalidated *Pachygrapsus socius*). These taxa are closely related and historically separated by the spread of the Atlantic seafloor and the closure of the Panama Isthmus. The comparison of the three disjunct populations or species in four genera with amphi-atlantic and trans-isthmian distribution allows to compare divergence patterns in consequence of the relatively fast and well-dated closure of the Panama Isthmus with those of a more gradual rupture of gene flow during the drifting apart of two continental plates (amphi-atlantic). In the present study, divergence rates of the 16S mtDNA gene are compared, allowing a relative time estimate of the loss of connectivity across the Atlantic, previously maintained by planktonic larvae.

2. Materials and methods

Specimens examined are deposited in museums as listed in Table 1. DNA sequences were obtained between 1994 and 2009 at the laboratories of the Pennsylvania State University (PSU), the University of Louisiana at Lafayette (ULL) (both USA) and the University of Regensburg (Germany). DNA extractions and selective amplification of 16S mitochondrial DNA were carried out as reported in Schubart et al. (1998, 2002, 2006a). Most PCR-amplifications were run with 4 min denaturation at 94 °C, 40 cycles with 45 s 94 °C, 1 min 48 °C, 1 min 72 °C and 10 min final elongation at 72 °C and the primers 16L2 or 16L29 and 16H2 or 16H10 (see Schubart, 2009). PCR products were purified with Microcon 100 filters (Microcon) or Quick-Clean (Bioline) and subsequently sequenced with the ABI BigDye terminator mix followed by electrophoresis in an ABI Prism 310 Genetic Analyzer (Applied Biosystems, Foster City, USA). New sequence data were submitted to the European molecular database EMBL (see Table 1 for accession numbers). In addition, the following 16S mtDNA ingroup sequences archived in molecular databases were included in this analysis: *G. lividus* Gulf of Mexico (AJ250651), *Goniopsis cruentata* Gulf of Mexico (AJ250652), *Grapsus grapsus* Gulf of Mexico (AJ250650), *Leptograpsus variegatus* Chile (AJ250654), *Metopograpus latifrons* Sabah, *Metopograpus quadridentatus* China, *Metopograpus thukuhar* Mozambique (AJ784028, DQ062732, AJ784028), *Pachygrapsus minutus* Japan, *P. socius* Peru, *P. transversus* Ibiza and Brazil (AB057808, AM180260, AM180256, FM539016) and *Planes minutus* Spain (AJ250653). As outgroups, the type species of Sesarmidae, *Sesarma reticulatum* (AJ225867) and Varunidae, *Varuna litterata* (AJ308419) as well as a representative of the monogeneric Xenograpsidae, *Xenograpsus testudinatus* (FM863827) were included. Sequences were manually aligned taking into account secondary structure (see Schubart et al., 2000).

The model of DNA substitution that fitted the data best was determined using the software MrModeltest (Nylander, 2004). For phylogenetic inference we applied Bayesian inference (BI) as implemented in MrBayes v. 3.1 (Huelsenbeck and Ronquist, 2001) and trees were calculated using the suggested model of evolution by MrModeltest. The Bayesian analysis was run with four MCMC (Markov chain Monte Carlo) chains for 4,000,000 generations, saving a tree every 500 generations (with a corresponding output of 8000 trees) and with *S. reticulatum* as defined outgroup. The $-\ln L$ converged on a stable value between 15,000 and 20,000 generations (burn-in phase), and the first 50,000 generations were subsequently excluded from the analysis before calculating the final tree. Posterior probabilities of the phylogeny were determined by constructing a 50% majority-rule consensus.

The mitochondrial dataset consisted of 42 sequences resulting in 580 aligned characters, of which 293 were conserved, 226 parsimony-informative and 280 variable. GTR + G + I was selected as the best-fitting evolutionary model by MrModeltest and implemented for subsequent Bayesian analysis. The resulting consensus tree with BI posterior probabilities is shown in Fig. 1.

3. Results

The Bayesian phylogenetic tree as shown in Fig. 1 indicates that all grapsid representatives, except for the presumed grapsid genus *Leptograpsodes*, are monophyletic with a maximal posterior probability (pp) support of 1. *Leptograpsodes octodentatus* on the other hand appears closely related to *X. testudinatus* (pp = 1), a representative of the recently erected family Xenograpsidae for thermal vent crabs of the genus *Xenograpsus*, previously included in the Varunidae.

The early splits within the Grapsidae are impossible to resolve on the basis of this analysis and therefore no conclusion will be drawn and discussed, except for the fact that there is an early radiation within the Grapsidae with the so far monophyletic genus *Metopograpsus* holding a basal posi-