Gondwana revisited: New insights in the phylogenetic relationships within the Orthalicoidea (Mollusca, Stylommatophora)

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Introduction
The land snail (super)family Orthalicidae (Orthalicoidea) is a large family distributed on most southern continents, but predominantly present in the Neotropics. Its current taxonomic status is under dispute, some authors (Schileyko, 1999; Neubert et al., 2009) downgrading or giving familiar rank to some of the groups previously treated as subfamilies (Breure, 1979) (see Figure 1). The phylogenetic relationships are not well understood, due to scant molecular studies in which a limited number of species from this group are treated (Wade et al., 2001; Parent & Crespi, 2006; Wade et al., 2006; Herbert & Mitchell, 2009; Ramirez et al., 2009; Trewick et al., 2009). Moreover, Uit de Weerd (2008) suggested a relationship of the Coelociontidae and Megaspiridae to this group.

Material and methods
In total 38 taxa were sampled, spanning all groups within the Orthalicoidea. Genomic DNA was extracted using a DNeasy Tissue Kit (Qiagen). Primers (LSU 1–4), reaction conditions and cycling parameters were obtained from Wade & Morgan, 2000. PCR-products were sequenced in both directions on an ABI3730 capillary sequencer by Macrogen Europe. Forward and Reverse sequences were assembled with Sequencher v.4.2 (Gene Codes corp.). Contig-sequences were aligned using CLUSTALW and ambiguously aligned regions were excluded, using MacClade 4.0.8, resulting in 969 bp for ITS2/28S. The model of sequence evolution was selected using jModeltest 0.1.1 (Posada, 2008). Neighbourhood-joining consensus (NJ), Bayesian inference (BI) and maximum likelihood (ML) analyses were executed using PhyML and MrBayes in Geneious 19.8. 4.3.8 (Drummond et al., 2009) and BEAST 1.5.3 (Drummond & Rambaut, 2007) with default settings and the model selected by jModeltest.

Results and discussion
All methods of tree construction employed (NJ, ML, BI) resulted in trees that were generally consistent, with a number of clades (Figure 2A-B). Clades that are referred by Bouchet et al. (2005) to Bulimulinae (Bulimus, Naesiotus A+B, Bostryx A+B, Drymaeus A+B) are strongly supported as a group (100/100/1 for bootstrap support and posterior probabilities), albeit with some less resolved relationships within the group. Two clades, Spixia and Plagioidantes, representing the Odontostominae of Breure (1979) are moderately supported as a group (95/94/0.97). The next group consists of Placostylus s.l. (corresponding to the Placostylidae of Bouchet et al., 2005), Bostrychomyia, Prestonella and Plectostylus. This group is moderately supported (88/95/1), but within the group support values vary. The Orthalicinae is a moderately supported clade (100/94/0.99), including some species hitherto classified within the Bulimulinae. Finally, there is a well supported group (100/98/1), consisting of Plekocheilus and Gaeotis. The latter was hitherto placed in the Amphibullimidae by Bouchet et al. (2005).

The results are generally consistent with the cladogram presented by Breure (1979), except for the Bulimulinae which appear to be paraphyletic. The placement of Coelociontidae in the superfamily is not supported by the current results, but needs further study. The results corroborate those of Herbert & Mitchell (2009) in placing Prestonella inside the Orthalicoidea. They suggested that little derived lineages occur in South Africa (Prestonella) and Australasia (Bostrychomyia), with the anatomically more derived Placostylidae occurring in Melanesia (Figure 2B). As shown in our results, the clades of Orthalicus s.l., Plekocheilus and Gaeotis may be considered more basal, while odontostomids and ‘true’ bulimulids may be considered more derived. Thus, the Gondwanan relationships between the different clades appear more complicated than hitherto supposed and warrant further research.