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A phylogeny of the cannibal snails of southern Africa, genus *Natalina sensu lato* (Pulmonata: Rhytididae): Assessing concordance between morphology and molecular data

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ABSTRACT

The genus *Natalina* Pilsbry, 1893 is a southern African endemic belonging to the Gondwanan family of carnivorous snails, Rhytididae. We present a well-resolved molecular phylogeny of the genus based on the mitochondrial 16S and COI genes and the nuclear ITS2 gene, and assess this in light of Watson's [Watson, H., 1934. *Natalina* and other South African snails. Proc. Malacol. Soc. Lond. 21, 150–193] supra-specific classification via a re-examination of 23 morphological characters including features of the shell, radula, external anatomy and distal reproductive tract. Ancestral reconstruction and character mapping based on the MK_1 model reveals broad concordance between morphology and the molecular phylogeny at the supra-specific level. Given this concordance and exceptionally deep divergences in the molecular data, we recommend the elevation of the subgenera *Natalina s.s., Afrorhytida*, and *Capitina* to generic status. At the species level, we identify several species complexes for which additional fine scale morphological and molecular appraisal is needed to qualify on the one hand incipient speciation with notable differentiation in shell form and body pigmentation, and on the other, phylogenetically deep yet morphologically cryptic diversity.

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1. Introduction

The terrestrial malacofauna of southern Africa is exceptionally diverse and exhibits a high degree of regional endemicity. Over 650 terrestrial mollusc species occur in the region south of the Cunene and Zambezi rivers, 90% of which are endemic (Bruggen, 1978; Herbert, 1998). A notable constituent of this endemicity is those species belonging to the southern relictual fauna, namely the families Bulimulidae, Chlamydephoridae, Dorcasiidae, Rhytididae and Sculptariidae. The cannibal snails of the family Rhytididae represent a charismatic component of this relictual fauna and include some of the largest carnivorous snails in the world. These snails are believed to be of Gondwanan origin, with further representatives of the family distributed in Australia, New Zealand and islands in Indonesia and the south-western Pacific (Bruggen, 1978; Solem, 1959). Two rhytidid genera are currently recognised in southern Africa, *Nata* Watson, 1934 and *Natalina* Pilsbry 1893, in which Connolly (1939) recognised six and 17 species, respectively, the latter confined to the more mesic southern and eastern parts of the region (Fig. 1). However, the species-level taxonomy of these genera is not well resolved and many uncertainties remain. As relatively large carnivores, population densities of *Natalina* species are concomitantly low and a number exhibit ranges of very limited extent. For these species, habitat fragmentation, degradation and loss are issues of major concern (Herbert, 1998). Two species, *Natalina beyrichi* (Transkei coast) and *N. wesseliana* (Zululand), are currently listed as critically endangered and vulnerable, respectively (IUCN, 2007), and others urgently need evaluation in this regard. To aid future assessment of the conservation status of these taxa, however, a revision of the taxonomy and systematics of southern African rhytidids is necessary. This paper represents the first contribution to this endeavour, providing a phylogenetic assessment of the genus *Natalina* (*sensu lato*).

We present a molecular phylogeny based on partial sequences of mitochondrial 16S and COI genes and the nuclear ribosomal gene ITS2, and assess this in light of Watson's (1934) morphological classification. Of particular interest is the supra-specific classification recognising three subgenera within *Natalina*, namely the monotypic subgenus *Capitina* Watson, 1934 accommodating the Western Cape endemic *Natalina schaerfiae* (Pfeiffer, 1861), *Natalina*

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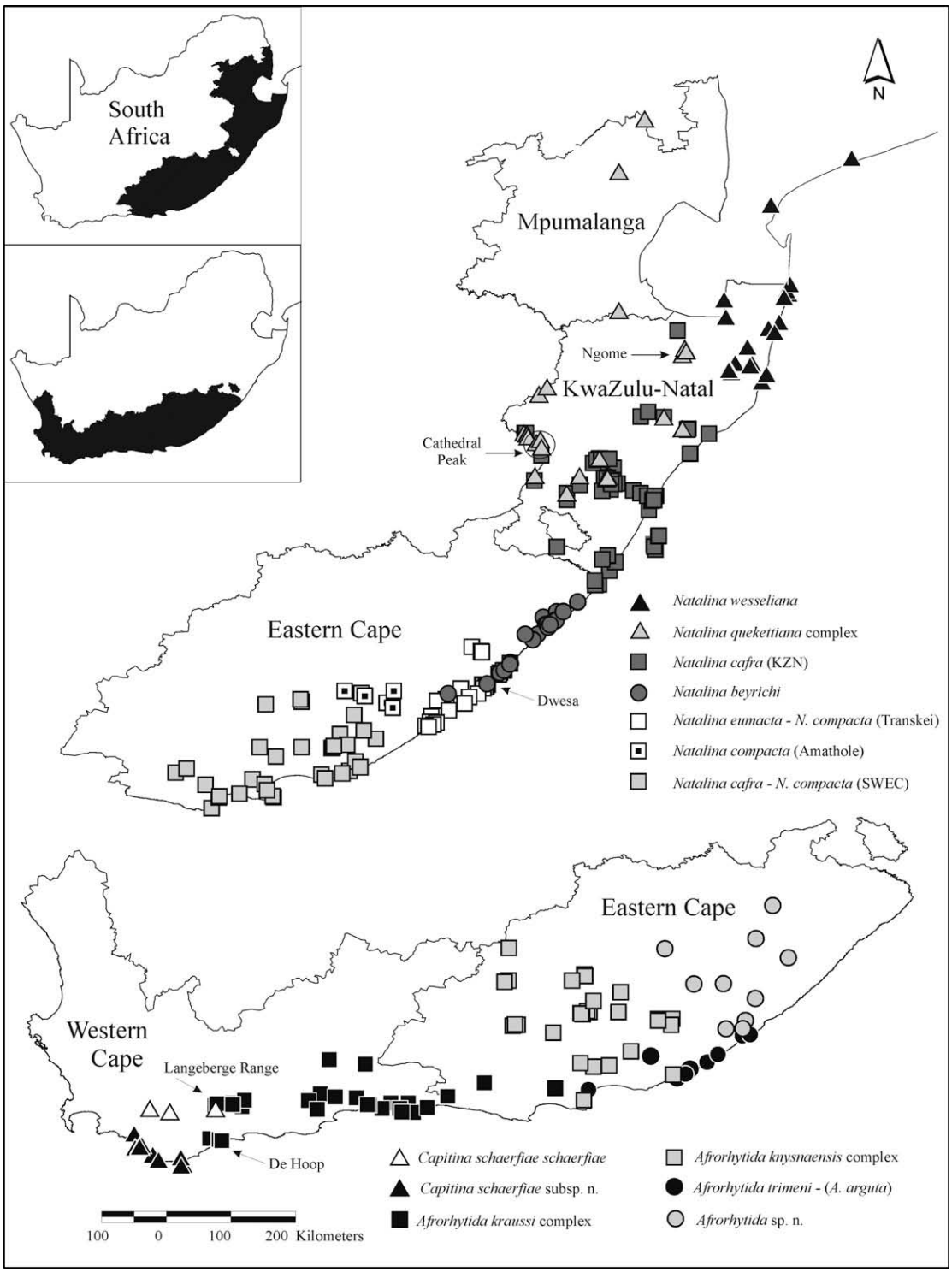

Fig. 1. Locality records of *Natalina s.l.* specimens held at the Natal Museum, Pietermaritzburg, South Africa. All vouchers were re-examined and the taxonomy verified via reference to sequenced specimens.

sensu stricto Pilsbry, 1893 and *Afrorhytida* Möllendorff, 1903. The latter subgenus was further subdivided into two sections, *Afrorhytida s.s.* and *Hyperrhytida* Watson, 1934. We re-evaluate this existing supra-specific taxonomy based on a more robust molecular phylogenetic framework and an examination of 23 morphological characters including features of the shell, radula, external anatomy and distal reproductive tract. We map these characters onto the molecular phylogeny and reconstruct ancestral character states to examine the evolution of these traits and assess their phylogenetic utility and concordance with the molecular data. Our reinterpretation of the species-level taxonomy in light of the genetic lineages identified, in conjunction with the morphological data, provides greater nomenclatural clarity and reveals the existence of a number of synonyms as well as several undescribed lineages. These new taxa will be formally named and described in a full taxonomic revision of the genus (Herbert and Moussalli, in preparation). By integrating molecular and morphological data to understand the systematics of *Natalina s.s.*, this study will contrib-

ute significantly to our ability to evaluate the conservation status of the species comprising this charismatic component of southern African invertebrate biodiversity.

2. Methods

2.1. Specimens examined

We sequenced a total of 62 individuals representing 14 of the 17 nominate species of *Natalina* (Appendix A). Tissue samples from the hind end of the foot were excised and preserved in 99% ethanol prior to the animal being preserved in 75% ethanol for anatomical study. For all species we attempted to ensure that at least one of the samples sequenced was collected at or in close proximity to the type locality, and were successful in the majority of cases. Species identification was confirmed with reference to type material. In a number of cases, however, we have been unable to discriminate consistently between the described nominate species and consider that some will eventually prove to be synonyms. These species are identified using the oldest available name for the species cluster. Thus in our phylogenetic analyses *N. knysnaensis* (Pfeiffer, 1846) includes *N. coerneyensis* Melvill and Ponsonby, 1894 and *N. insignis* Melvill and Ponsonby, 1907, and similarly *N. kraussi* (Pfeiffer, 1846) includes *N. liliacea* Preston, 1912. Despite repeated survey attempts, we were unable to obtain specimens of *N. reenenensis* Connolly, 1939 and *N. inhluzana* (Melvill and Ponsonby, 1894) from the respective type localities, primarily due to loss of habitat. Nevertheless, specimens showing considerable affinity to the *N. reenenensis* type material were obtained from the Drakensberg, within the Cathedral Peak region (see Fig. 1). In regard to *N. inhluzana*, based on a comparison of the type material (Natural History Museum, London (NHM) and Natal Museum, Pietermaritzburg, South Africa (NMSA)) and geographic proximity (approximately 35 km), this species is tentatively considered to be synonymous with *N. quekettiana* (Melvill and Ponsonby, 1893). Conversely, in his discussion of *N. schaerfiae*, Watson noted a potential second allied species, and we were successful in obtaining live material of this taxon. Preliminary morphological examination of this taxon confirms its distinctiveness (Herbert and Moussalli, unpublished data) and we include it here for completeness. Lastly, a further species recognised by Connolly (1939), namely *N. caffrula* Melvill and Ponsonby, 1898, is not considered here. Examination of type specimens of this species (NHM and South African Museum) shows it to possess a small (diam. <2.5 mm), smooth and glossy embryonic shell and thus to be referrable to the genus *Nata* rather than *Natalina*. To root the molecular phylogeny, we sequenced four outgroup taxa, two species of *Nata*, *N. vernicosa* (Krauss, 1848) and *N. tarachodes* (Connolly, 1912), and two Australian rhytidids, *Prolesophanta dyeri* (Petterd, 1879) and *Strangesta strangei* (Pfeiffer, 1849). Vouchers of all South African specimens used in this study have been deposited at the NMSA, while the two Australian representatives are deposited at Museum Victoria, Australia.

2.2. Laboratory procedures

We extracted total genomic DNA using the ammonium acetate procedure described in Moussalli et al. (2005). Small pieces of foot muscle tissue were first digested at 37 °C overnight in a buffer solution containing Proteinase K. Proteins and cell debris were then removed by precipitation using 4 M ammonium acetate, followed by purification using ethanol precipitation. For amplification of approximately 950 bp of COI we used the universal invertebrate forward primer LCO1490 (Folmer et al., 1994) and the reverse primer H7005-mod1 of Donald et al. (2005), while for ITS2 (approximately 450 bp) we used the forward and reverse primers of Wade and Mordan (2000), LSU-1 and LSU-3, respectively. For 16S, we amplified approximately 900 base-pairs using the primers 16Smh1: 5′TTTGTACCTTTTGCATAATGG3′ and 16Smh2: 5′TAAGGT CCTTTCGTACTA3′, modified from Chiba (1999). PCR cycles were as follows: initial denaturation 94 °C for 2 min; 35 cycles of 94 °C for 45 s, 50–54 °C (CO1 and 16S) or 60–64 °C (ITS2) for 1 min, 72 °C for 1.5 min; final extension 72 °C for 5 min. PCR products were purified by ethanol precipitation with sequence reactions and capillary separation done by Macrogen Inc., Seoul, Korea. Nucleotide sequences were first aligned using CLUSTAL W (Thompson et al., 1994) followed by manual appraisal. The COI protein coding region was checked against translated amino acid sequences using MEGA version 4 (Tamura et al., 2007), while 16S and ITS2 alignments were checked against secondary structure models as described in Lydeard et al. (2000) and Schultz et al. (2008), respectively. Sequences are lodged with GenBank, accession numbers: FJ262172–FJ262365.

2.3. Molecular analysis

We conducted the phylogenetic analysis using MrBayes version 3.1.2 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003), with model selection determined using MrModelTest (Nylander, 2004). This was done for each gene separately, for a combined mtDNA dataset and for a combined dataset incorporating all three genes. Multi-gene datasets were partitioned and analysed such that models of sequence evolution were parameterised separately for each partition except branch length estimation. In the case of the ITS2 dataset we also explored the phylogenetic signal in structural variation via binary coding of insertions and deletions (see Ronquist et al., 2005). The binary dataset was assessed concurrently with the nucleotide alignment in a partitioned analysis using the restriction site model and variable coding bias (from here on referred to as the ITS2 + structural model). For all analyses, we performed two simultaneous runs with convergence determined by assessing the average standard deviation of split frequencies (<0.01). We examined the degree of congruence among the different genes (16S, COI and ITS2) using the incongruence-length difference test in PAUP (Farris et al., 1995) with invariant sites removed. Additionally, we assessed each of the single gene reconstructions for topological congruence with phylogenetic relationships resolved by the combined dataset analysis. Specifically, we derived a 95% credible set of unique topologies (using tree probability files obtained from the SUMT procedure in MrBayes) for each of the three single gene analyses. These were then screened for topological hypotheses using the CONSTRAINTS and FILTER functions in PAUP (Swofford, 2001). The occurrence of the topological hypothesis within the 95% credible set of trees indicates that "the topology could have given rise to that data" (Buckley et al., 2002).

2.4. Morphological assessment

We examined morphological characters from a suite of four anatomical systems. These included features of the shell, radula, external anatomy and distal reproductive tract. In total, we assessed 23 characters (Table 1) and a discussion thereof, together with character state coding is provided in Appendix A. The primary source for this data was Watson (1934), together with supplementary information from earlier single species studies (Pace, 1895; Pilsbry, 1889, 1890; Woodward, 1895). However, in all cases we confirmed morphological data via our own observations since species discrimination in some of these earlier studies is suspect and/or the data were based on only one specimen. For some species, details of morphology were not previously available, necessitating *de*

Table 1
Morphological dataset. Refer to Appendix B for character description and scoring.

	Embryonic shell diameter	Embryonic shell sculpture	Peristome	Left mantle lobes	Skin Texture	Labial palps	Genital pore	Radula – transverse rows	Rachidian tooth	Latero-marginal transition	No. lateral teeth	No. of marginal teeth	Ratio laterals: marginals	Inner lateral teeth-shape	Penis position	Penis shape	Penis/ epiphallus lower vas deferens	Epiphallus length	vaginal length	Duct of bursa copulatrix length	Duct of bursa copulatrix position	Sperm-oviduct	Oviduct caecum
	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)	(16)	(17)	(18)	(19)	(20)	(21)	(22)	(23)
Capitina schaerfiae[a] [5]	1	1	1	3	0	1	1	1	1	3	NA	NA	NA	0	1	1	1	2	1	1	0	1	0
C. schaerfiae subsp. n. [2]	1	1	1	3	0	1	1	1	1	3	NA	NA	NA	0	1	1	1	2	1	1	0	1	0
Afrorhytida knysnaensis [6]	1	1	1	1	0	1	1	1	1	1	0	1	2	0	1	1	2	1	1	1	0	1	1
A. kraussi [3]	1	1	1	1	0	1	1	1	1	0	1	3	3	0	1	1	2	1	1	1	0	1	0
A. kraussi (De Hoop) [1]	1	1	1	1	0	1	1	1	1	0	1	3	3	0	1	1	2	1	1	1	0	1	0
A. trimeni [2]	1	1	1	1	0	1	1	1	1	0	1	2	0	1	1	1	2	1	1	1	0	1	1
A. sp. n. [1]	1	1	1	1	0	1	1	1	1	0	1	2	0	0	1	1	2	1	1	1	0	1	0
Natalina cafra subsp. n. (KZN) [3]	2	1	0	1	0	1	1	1	1	2	0	1	1	0	0	1	1	1	1	1	1	1	2
N. cafra cafra (SWEC) [5]	2	1	0	1	0	1	1	1	1	2	0	1	1	0	0	1	1	1	1	1	1	1	2
N. cafra subsp. n. (Amathole) [1]	2	1	0	1	0	1	1	1	1	2	0	1	1	0	0	1	1	1	1	1	1	1	2
N. eumacta (Transkei) [3]	2	1	0	1	0	1	1	1	1	2	0	1	2	0	0	1	1	1	1	1	1	1	2
N. compacta (Transkei) [3]	2	1	0	1	0	1	1	1	1	2	0	1	2	0	0	1	1	1	1	1	1	1	2
N. beyrichi [1]	2	1	0	1	0	1	1	1	1	2	0	1	1	0	0	1	1	1	1	1	1	1	2
N. quekettiana [4]	1	1	1	1	0	1	1	1	1	2	0	3	2	0	0	1	1	1	1	1	1	1	2
N. quekettiana (Injasuthi) [1]	1	1	1	1	0	1	1	1	1	2	0	3	2	0	0	1	1	1	1	1	1	1	2
N. quekettiana (Cathedral Peak) [1]	1	1	1	1	0	1	1	1	1	2	0	3	2	0	0	1	1	1	1	1	1	1	2
N. quekettiana (Ngome) [1]	1	1	1	1	0	1	1	1	1	2	0	3	2	0	0	1	1	1	1	1	1	1	2
N. wesseliana [1]	2	1	0	2	1	1	3	1	1	2	0	1	1	0	0	1	1	1	1	1	1	1	2
Nata tarachodes [1]	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Nata vernicosa [8]	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

[a] Number of specimens examined per taxon given in square brackets.

novo observation. Where possible, we assessed morphological characters for at least three specimens of each species, including, in all cases, one or more of the specimens sequenced. To assess concordance between the morphological and molecular data we mapped each morphological character onto the combined molecular phylogeny with ancestral reconstruction done using the MK_1 model as implemented in MESQUITE ver. 2.5 (Maddison and Maddison, 2008). Only unequivocal ancestral state reconstructions are presented, with the likelihood decision threshold set to the default of two log likelihood units.

3. Results

Sequence alignment for COI was unambiguous as no length variation or premature stop codons were observed, consistent with the assumption that sequences represent functional copies that encode a protein. The secondary structure of both 16S and ITS2 conformed to that documented for eukaryotes (De Rijk et al., 1999; Schultz et al., 2008, respectively). For 16S, the partial sequences included Domains II, IV and V (see Lydeard et al., 2000 for Domain delineation), with the first Domain exhibiting the greatest degree of structural variability (~25% alignable) and Domain IV the least (~70% alignable). All regions of ambiguous alignment were removed prior to phylogenetic analysis. For ITS2, all ingroup taxa were consistent with the general eukaryote structure in having four helices, a pyrimidine-pyrimidine mismatch in helix II, and helix III being the longest and containing the conserved UGGU motif (see Coleman, 2007; Schultz et al., 2008). Ingroup sequence length for ITS2 ranged between 379 – 469 bp, with most regions of insertions and deletions alignable. Two hyper-variable regions found distally in Helix II and basally in Helix III, however, were unalignable across the ingroup and were removed. Additionally, approximately 50% of the outgroup ITS2 sequences were not unambiguously alignable with the ingroup. To maximise information contained within the ingroup, we included these ambiguously aligned sites by coding them as unknown for the outgroup taxa. In total, 1944 nucleotide positions (COI = 1076, 16S = 401, ITS2 = 465) were unambiguously aligned, of which 782 were variable and 632 phylogenetically informative. The GTR model with site heterogeneity (Γ) and proportion of invariant sites (I) parameters was chosen as the best AIC model for both 16S and COI, while the GTR + I was chosen as the best fit for ITS2.

3.1. Molecular analysis

There was no significant incongruence between the three gene partitions based on the ILD test (16S + COI, $p = 0.90$; 16S + ITS2; $p = 0.61$, COI + ITS2, $p = 0.99$). Consistent across all datasets (single genes and combined) are three highly divergent lineages within *Natalina s.l.* (Figs. 2 and 3), a pattern consistent with the subgeneric classification proposed by Watson (1934), namely the three taxa *Natalina s.s.*, *Afrorhytida* and *Capitina*. At this subgeneric level, phylogenetic resolution varied considerably between the three molecular datasets, with ITS2 exhibiting the greatest resolution and the mitochondrial datasets the least (single gene mitochondrial phylograms are provided in Supplementary Material). This is essentially due to ITS2 being conserved relative to the mitochondrial genes, a fact reinforced by the lack of genetic differentiation based on ITS2 nucleotide data within species complexes such as the *N. quekettiana* or *N. kraussi* complexes (Table 3). Accordingly, the monophyly of *Natalina s.l.* and the sister relationship of *Afrorhytida* and *Natalina s.s.*, are well supported only in the ITS2 and combined data (all three genes) reconstructions (Figs. 2 and 3). However, in the partitioned ITS2 + structure analysis, although support for the monophyly of *Natalina s.l.* is maintained, phylogenetic relationships among the three subgenera are unresolved, suggesting that structural variability (insertions and deletions) at this phylogenetic level is non-homologous. Nevertheless, there was no significant discordance for these deeper relationships (topological hypotheses A–C in Table 2) in either the single gene mitochondrial or the ITS2 + structure analysis based on the 95% credible set of topologies.

Mean observed and estimated divergence between and within taxa are given in Table 3. For the sake of clarity, only estimated percent sequence divergences are given in the text. Sequence divergences between the three major lineages within *Natalina s.l.* are comparable and considerable. Based on 16S, maximum mean

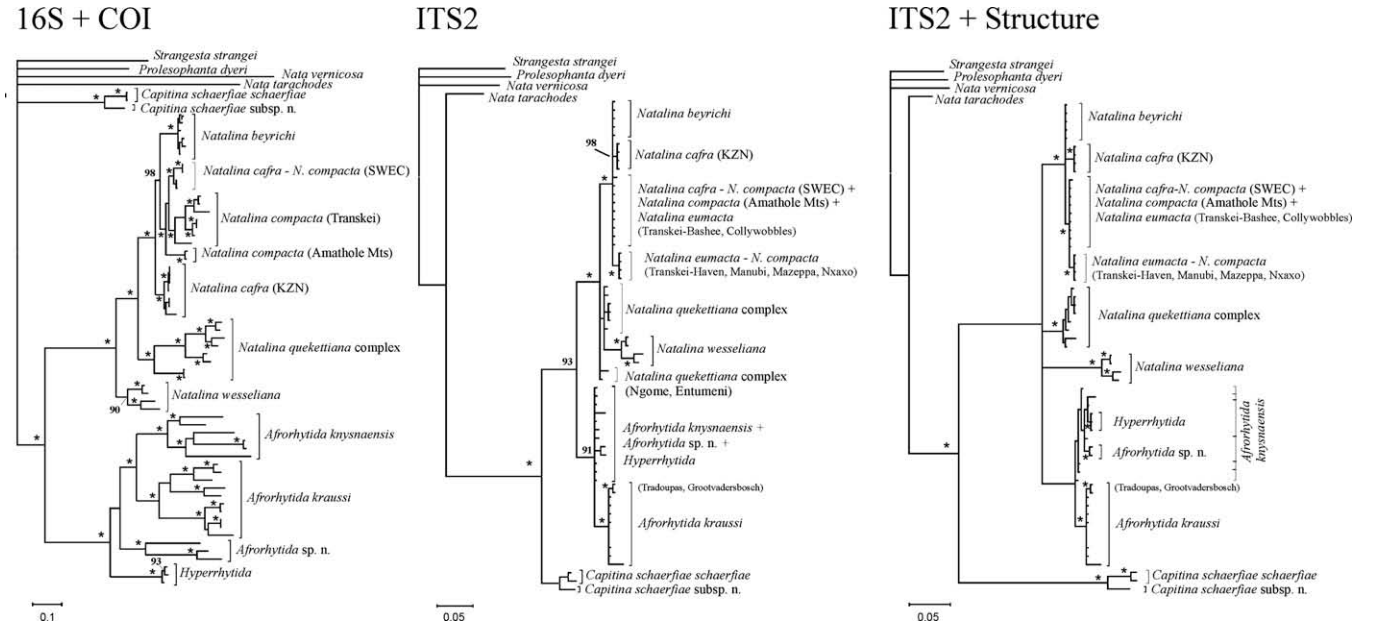

Fig. 2. Fifty percent majority rule consensus trees for, from left to right, 16S and COI combined (both GTR + IΓ), ITS2 (GTR + I) and ITS2 and INDELS combined (GTR + I and Restriction Site Model with coding bias being variable, respectively). Asterisk indicates nodal support >95% posterior probability (PP). Nodal support between 90 and 95% PP are given explicitly, while PP below 90% are not shown.

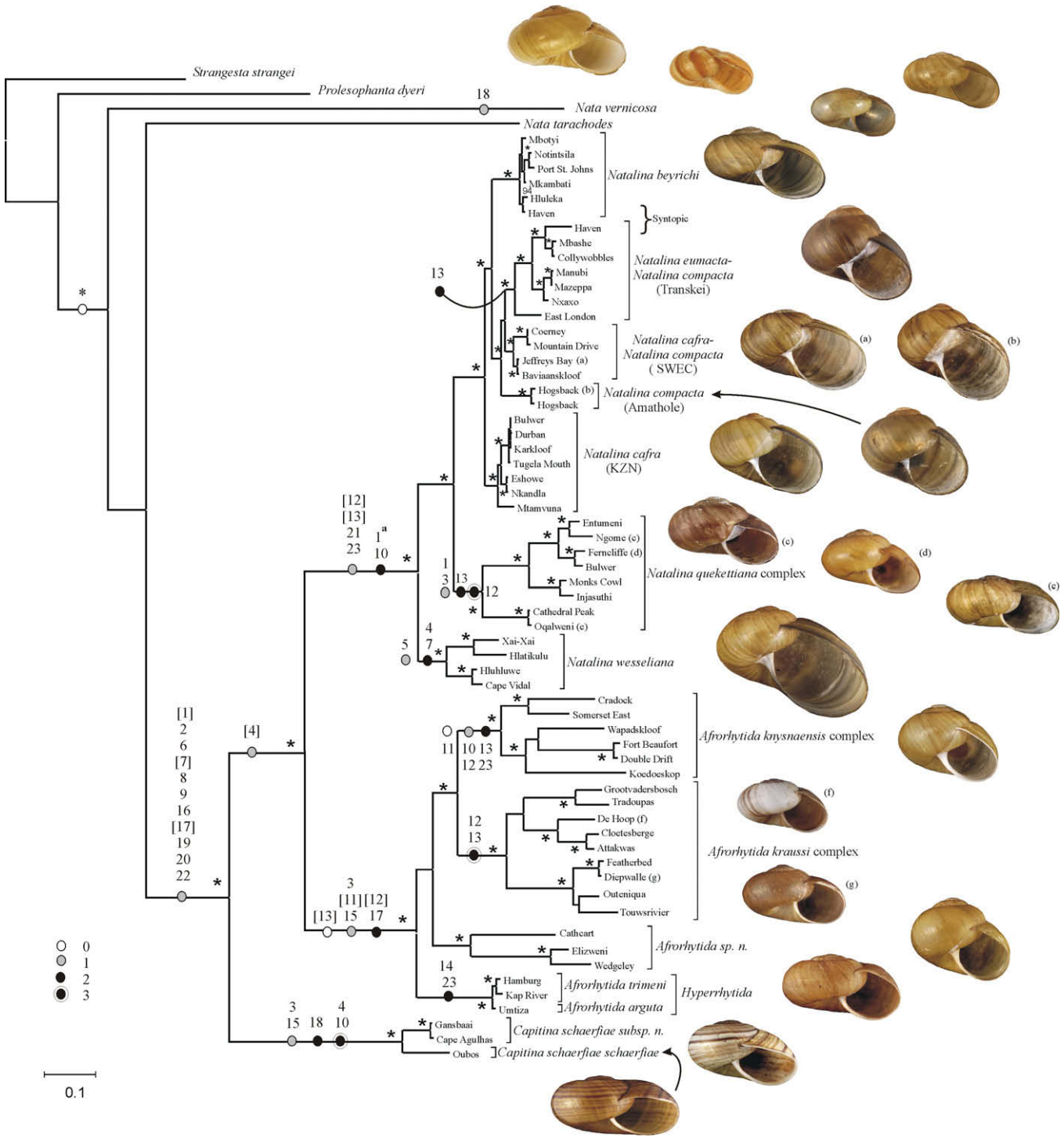

Fig. 3. All compatible Bayesian consensus tree based on mixed model partitioned analysis of the combined nucleotide dataset (16S and COI = GTR + IΓ, ITS2 = GTR + I). Ancestral reconstructions are summarised for those nodes having unequivocal state changes (refer to Appendix B for character descriptions and scoring). Note, to aid presentation of the character mapping, character states for the South African outgroup taxa, *Nata vernicosa* and *Nata tarachodes* were assigned the 'zero' (open circle). Additionally, characters in square brackets indicate characters with subsequent state changes, while non-bracketed characters indicate synapomorphies. Asterisk indicates nodal support >95% posterior probability (PP). Nodal support between 90–95% PP are given explicitly, while PP below 90% are not shown. Representative shell images are given for each clade. In some instances, multiple representatives are given. These have been labelled (a–g) next to both the shell and the respective specimen/ population in the tree.

divergence was as high as 95% (GTR − I Γ) between the *N. compacta*/*N. eumacta* (Transkei–East London) clade and the *A. knysnaensis* complex. Similar levels of divergence are observed in COI, ranging from a maximum of 130% (GTR − I Γ) for the same pair of taxa, to a minimum of 15% between the south-western 'E. Cape' clade within *Natalina s.s.* (*N. cafra*/*N. compacta* (SWEC)) and *N. compacta* (Amathole) (see Fig. 3). The mtDNA dataset exhibits strong saturation (Fig. 4), however, so large error bounds in branch length estimates are expected at deeper divergences. Conversely, no saturation is evident in the relatively conserved ITS2 dataset. Based on this gene, there was a maximum of 18% (GTR − I) mean divergence between *N. wesseliana* and *Capitina schaerfiae*.

Table 2
Test of single gene analysis for topological hypotheses representing resolved phylogenetic relationships based on the combined, partitioned analysis (16S + COI + ITS2).

	16S	COI	ITS	ITS2 + structure
Total number of trees within 95% confidence set	8829	6835	42,774	57,030
Topological hypotheses[a]				
A = (*outgroup* (*Natalina s.l.*))	0.14	0.17	1.00	0.95
B = (*Capitina*,(*Afrorhytida*),(*Natalina*))	0.52	0.75	0.45	0.39
C = ((*Afrorhytida*),(*Natalina*))	1.00	1.00	0.91	0.39
D = ((A. *trimeni* +*A. sp. nov*),(A. *knysnaensis*),(A. *kraussi*))	0.09	0.98	0	0
E = ((N. *wesseliana*, (N. *quekettiana* complex),(cafa- group))	0.61	0.76	0.09	0
F = ((N. *cafra* 'KZN'),(N. *beyrichi*),(N. *cafra* 'E. Cape' + *N. compacta*))	0.30	0.91	0	0.01
G = ((N. *cafra* '*KZN*'+*N.cafra* E. Cape) remaining *Natalina s.s*))	0	0	0	0

[a] For each single gene analysis a 95% confidence set of unique topologies is derived by removing topologies greater than 0.95 cumulative posterior probability the tree probably file produced from the SUMT procedure in MrBayes. The confidence set is then screened for the 7 topological hypotheses (A–Z) using the 'constraints' and 'filter' functions in PAUP. Results are presented as percentages.

Within *Natalina s.s.*, all datasets are consistent in delineating three distinct clades: namely *Natalina wesseliana*, the *Natalina quekettiana* complex of small Afrotemperate taxa, and thirdly, a group of relatively large species that have traditionally been identified as *N. cafra*, *N. beyrichi*, *N. compacta* and *N. eumacta* (from here on referred to as the *cafra*-group) (Figs. 2 and 3). Only the mitochondrial datasets, however, resolve the sister relationship between the latter two clades. 16S sequence divergences between representatives of the three major clades within *Natalina s.s.* ranged from 8% between the *N. cafra*/*N. compacta* (SWEC) clade and *N. compacta* (Amathole) to 30% between *N. wesseliana* and *N. quekettiana* complex (Table 3). In comparison, for the same pairs of taxa, COI divergences were 15 and 56%, respectively whereas ITS2 divergences were 0 and 6%. Based on the mtDNA datasets, mean within-group divergences were comparable in *N. wesseliana*, the *N. quekettiana* complex and the *cafra*-group (e.g.16S (GTR − I Γ) – 9, 14 and 11%, respectively, Table 3).

For the *cafra*-group, there is strong geographic structure, which does not coincide with the current taxonomy. Firstly, *N. cafra s.l.* is paraphyletic, with the populations comprising topotypic material of *N. cafra*, *N. compacta*, *N. eumacta* and *N. beyrichi* forming a monophyletic clade distinct from the more northerly KwaZulu-Natal population of *N. cafra* (henceforth referred to as *N. cafra* KZN). This relationship is strongly supported only by the mitochondrial datasets (Fig. 2a; hypothesis F, Table 2), with 14 and 22% mean divergence (16S and COI, respectively) separating *N. cafra* KZN and its sister clade. The ITS2 dataset does support, however, the monophyly of *N. cafra* (KZN), and under the ITS2 + structure model, the paraphyly of *N. cafra s.l.* is not rejected based on the 95% confidence set (hypothesis F, Table 2). Further, we find no support from either the mitochondrial or the nuclear datasets for the alternative topological hypothesis of a monophyletic *Natalina cafra s.l.* (hypothesis G, Table 2). Secondly, there are three clades within the E. Cape province inconsistent with current taxonomy, namely, (1) a south-western clade (SWEC) of very variable shell proportions which includes topotypic material of both *N. cafra* and *N. compacta*; (2) a primarily coastal clade from East London and Transkei with consistently globose shells including both topotypic material of *N. eumacta* and specimens conforming to typical *N. compacta* and (3) a clade of highly globose specimens identified as *N. compacta* from the Amathole region. Maximum sequence divergence between the latter two clades is 10 and 25% for 16S and COI, respectively.

Within the *N. quekettiana* complex, there are four distinct lineages based on the mtDNA dataset. Two of these lineages are restricted to the central KwaZulu-Natal Drakensberg (Cathedral Peak–Injasuthi, Fig. 3) and exhibit high sequence divergence (e.g. 6% based on 16S). The two remaining lineages are sister taxa and occur at lower altitude in mistbelt and scarp forests of KwaZulu-Natal (one includes topotypic material of *N. quekettiana s.s.* from Ferncliffe, Pietermaritzburg, Fig. 3). This pair forms a sister relationship with one of the Drakensberg lineages, specifically that representing the Monks Cowl and Injasuthi populations (12% 16S sequence divergence).

Based on the combined analysis, there are four well supported clades within *Afrorhytida*, representing the *A. knysnaensis* complex, the *A. kraussi* complex, the section *Hyperrhytida* (*A. trimeni* and *A. arguta*), and an undescribed species or complex. Resolution of phylogenetic relationships within *Afrorhytida*, however, is restricted to the sister relationship between the *A. knysnaensis* and *A. kraussi* complexes and is based principally on the mitochondrial dataset (hypothesis D, Table 3). In terms of ITS2, only the monophyly of the *A. kraussi* complex and a subclade within this representing isolated western populations in the forests of the western Langeberge (Tradoupas and Grootvadersbosch) is supported under the nucleotide model (refer to Figs. 2 and 3). Additionally, in the ITS2 + structure analysis, the monophyly of both *Hyperrhytida* and members of the undescribed lineage from East London and the Kei River catchment (Elizweni and Wedgeley populations) are also well supported.

Mean sequence divergences between representatives of the four *Afrorhytida* clades were comparable (Table 3), with a maximum of 39% (16S) and 18% (COI) between the undescribed lineage and *A. knysnaensis*. For ITS2, there was a maximum of 6.3% divergence between the *A. kraussi* and *A. knysnaensis* complexes. Mean within-group estimated mtDNA divergences varied, with the *Hyperrhytida* section (*A. trimeni* and *A. arguta*) exhibiting least diversity (2.8 and 2.9% for 16S and COI, respectively), while both the *A. knysnaensis* complex (19 and 50%, respectively) and the un-named lineage (16 and 44%, respectively) exhibited the greatest. For *A. knysnaensis*, however, there is no discernable geographic structure, while for the new lineage, the three sequenced individuals all come from the East London area and Kei River catchment, albeit at greatly varying altitudes. By contrast, the *A. kraussi* complex exhibits strong geographic structure in conjunction with relatively high within-group mean divergences (e.g. 16% for 16S). Of the populations examined, there are three subclades based on the mitochondrial dataset. The first represents populations primarily restricted to southern Afrotemperate forest in the Wilderness–Knysna region (e.g. Featherbed Nature Reserve); the second, with the exception of the coastal population at De Hoop, comprises populations found in montane mesic fynbos habitat, and the third, a clade found in western Afrotemperate forest isolates (e.g. Grootvadersbosch). Mean sequence divergences between the three clades were comparable and exceptionally high, with the greatest being between the two forest-restricted populations (Wilderness–Knysna and Grootvadersbosch–Tradoupas; e.g. 20% 16S sequence divergence).

Table 3
Mean sequence divergence for each single gene analysis, respectively.

	Outgroup	Nwe	Nca (KZN)	Nbe	Nco/Neu (Transksei)	Nco (Amathole)	Nca/Nco (SWEC)	Nqu complex	A (H)	A sp. n.	Akny	Aku	Ca
16S[a,b,c]													
Outgroup		0.174	0.177	0.180	0.179	0.172	0.178	0.183	0.184	0.185	0.198	0.188	0.171
Nwe	0.963	**0.090**	0.066	0.077	0.072	0.062	0.069	0.084	0.153	0.158	0.158	0.141	0.145
Nca (KZN)	1.022	0.217	**0.024**	0.036	0.039	0.030	0.034	0.067	0.157	0.155	0.166	0.144	0.147
Nbe	1.047	0.243	0.118	**0.025**	0.041	0.037	0.034	0.069	0.161	0.155	0.175	0.153	0.141
Nco/Neu (Transksei)	1.076	0.271	0.146	0.149	**0.063**	0.033	0.026	0.074	0.165	0.160	0.171	0.151	0.139
Nco (Amathole)	1.028	0.224	0.099	0.102	0.107	**0.014**	0.030	0.066	0.154	0.148	0.160	0.139	0.139
Nca/Nco (SWEC)	1.069	0.264	0.139	0.142	0.080	0.100	**0.029**	0.069	0.164	0.160	0.166	0.151	0.141
Nqu complex	1.096	0.292	0.223	0.249	0.277	0.230	0.270	**0.140**	0.162	0.158	0.164	0.147	0.146
A (H)	1.080	0.685	0.743	0.769	0.797	0.750	0.790	0.818	**0.028**	0.077	0.091	0.084	0.146
A sp. n.	1.005	0.610	0.668	0.694	0.722	0.675	0.715	0.742	0.233	**0.155**	0.113	0.094	0.145
Akny	1.232	0.836	0.895	0.920	0.949	0.902	0.942	0.969	0.316	0.385	**0.192**	0.093	0.168
Akr	1.194	0.798	0.857	0.882	0.911	0.864	0.904	0.931	0.278	0.347	0.315	**0.149**	0.150
Ca	0.872	0.650	0.708	0.734	0.762	0.715	0.756	0.783	0.767	0.692	0.919	0.881	**0.068**
COI													
Outgroup		0.194	0.202	0.195	0.204	0.201	0.199	0.203	0.191	0.211	0.200	0.201	0.195
Nwe	1.244	**0.171**	0.115	0.108	0.129	0.123	0.113	0.137	0.149	0.178	0.162	0.166	0.165
Nca (KZN)	1.288	0.401	**0.046**	0.072	0.099	0.088	0.070	0.130	0.154	0.185	0.170	0.171	0.169
Nbe	1.346	0.460	0.181	**0.037**	0.097	0.093	0.073	0.126	0.150	0.175	0.165	0.160	0.161
Nco/Neu (Transksei)	1.430	0.543	0.264	0.278	**0.120**	0.099	0.078	0.141	0.165	0.193	0.174	0.177	0.171
Nco (Amathole)	1.375	0.488	0.209	0.223	0.249	**0.018**	0.081	0.142	0.152	0.188	0.168	0.169	0.173
Nca/Nco (SWEC)	1.336	0.449	0.170	0.183	0.185	0.154	**0.039**	0.130	0.148	0.180	0.165	0.165	0.160
Nqu complex	1.442	0.556	0.452	0.510	0.593	0.538	0.499	**0.263**	0.166	0.188	0.175	0.175	0.179
A (H)	1.250	0.835	0.879	0.937	1.020	0.965	0.926	1.033	**0.029**	0.159	0.143	0.141	0.160
A sp. n.	1.468	1.052	1.097	1.155	1.238	1.183	1.144	1.251	0.778	**0.446**	0.170	0.169	0.193
Akny	1.509	1.094	1.138	1.196	1.279	1.224	1.185	1.292	0.819	0.887	**0.492**	0.158	0.176
Akr	1.528	1.113	1.157	1.215	1.298	1.243	1.204	1.311	0.838	0.906	0.868	**0.443**	0.174
Ca	1.196	1.013	1.057	1.116	1.199	1.144	1.105	1.212	1.020	1.238	1.279	1.298	**0.122**
ITS2													
Outgroup		0.139	0.129	0.127	0.130	0.127	0.127	0.135	0.121	0.115	0.125	0.139	0.117
Nwe	0.396	**0.029**	0.027	0.026	0.027	0.026	0.026	0.026	0.048	0.057	0.049	0.062	0.062
Nca (KZN)	0.391	0.103	**0.01**	0.002	0.005	0.002	0.002	0.016	0.044	0.049	0.046	0.058	0.059
Nbe	0.375	0.088	0.029	**0.02**	0.003	0.000	0.000	0.017	0.041	0.047	0.044	0.055	0.058
Nco/Neu (Transksei)	0.380	0.093	0.041	0.029	**0.028**	0.003	0.002	0.020	0.043	0.048	0.045	0.057	0.060
Nco (Amathole)	0.378	0.090	0.031	0.019	0.030	**0.027**	0.000	0.017	0.041	0.047	0.044	0.055	0.058
Nca/Nco (SWEC)	0.376	0.088	0.033	0.021	0.029	0.021	**0.23**	0.017	0.041	0.046	0.043	0.055	0.058
Nqu complex	0.371	0.063	0.078	0.063	0.068	0.065	0.063	**0.027**	0.040	0.047	0.042	0.054	0.056
A (H)	0.351	0.128	0.123	0.108	0.113	0.111	0.108	0.104	**0.015**	0.004	0.002	0.014	0.048
A sp. n.	0.361	0.138	0.133	0.118	0.123	0.120	0.118	0.113	0.025	**0.028**	0.007	0.016	0.054
Akny	0.356	0.133	0.128	0.113	0.118	0.116	0.113	0.108	0.024	0.032	**0.029**	0.016	0.050
Akr	0.371	0.148	0.143	0.128	0.133	0.130	0.128	0.123	0.053	0.063	0.058	**0.022**	0.058
Ca	0.306	0.175	0.170	0.154	0.160	0.157	0.155	0.150	0.130	0.140	0.135	0.150	**0.02**

[a] Taxa are as delineated in the combined data Bayesian phylogram (see Fig. 3).
[b] Within taxon mean uncorrected P-distances, estimated using the MEGA 4.0 (Tamura et al., 2007), are given along diagonal in bold; between taxa mean uncorrected P-distances are given above diagonal. Estimated divergences (16S and COI based on GTR + IΓ, ITS2 based on GTR + I) are given below diagonal.
[c] Taxa abbreviations are as follows: Nwe = *N. wesseliana*, Nca = *N. cafra*, Nco = *N. compacta*, Neu = *N. eumacta* Nqu = *N. quekettiana*, A (H) = *Afrorhytida* section *Hyperrhytida* = A. *trimeni*–*A. arguta* , Akny = *A. knysnaensis*, Akr = *A. kraussi* and Ca = *Capitina*.

3.2. Morphological assessment

Morphological character mapping and ancestral reconstruction revealed broad concordance between the morphology and the molecular phylogeny. These ancestral reconstructions need to be interpreted cautiously, however, as only two *Nata* species were examined as outgroup taxa. Relative to the genus *Nata*, eight synapomorphies are consistent with the monophyly of *Natalina s.l.* (Fig. 3), namely axially ribbed embryonic shell sculpture (character 2), well developed labial palps (6), greater than 40 transverse rows of radula teeth (8), the presence of a rachidian tooth (9), relatively long, cylindrical penis (16), long vagina (19) and bursa copulatrix duct (20), and a highly convoluted sperm-oviduct (22) (Fig. 3). Additionally, the monophyly of *Natalina s.l.* is supported by unequivocal reconstructions of apomorphic states in three characters for the common ancestor of the group, namely the embryonic shell diameter being intermediate in size (2.5–7.0 mm) (1), the positioning of the genital pore being ventral and just posterior to optic tentacle (7) and the absence of an oviduct caecum (23). These three characters, however, have undergone subsequent character evolution within *Natalina s.l.* (Fig. 3). First, there is marginal support (a threshold of 1.6-log likelihood) for an increase in embryonic shell size (>7 mm), thus overall size, defining *Natalina s.s.* with a subsequent reversal limited to the *N. quekettiana* complex. This pattern of character evolution is also reflected in the thickening of the outer lip of the shell at maturity (3), a character exhibited in all three subgenera with the exception of the *cafra*-group and *N. wesseliana*. In the latter case, the lack of thickening is shared with the outgroup, resulting in equivocal ancestral reconstructions. Secondly, the genital pore position shifts to well posterior the optic tentacle as an autapomorphic character defining *N. wesseliana.* Lastly, the presence of an oviduct caecum (23) has been independently derived on three occasions within *Natalina s.l.*: as a well developed caecum within *Natalina s.s.* and as a relatively small caecum derived on two separate occasions within *Afrorhytida*, in *A. knysnaensis* and *A. trimeni*.

In terms of the sister relationship between the subgenera *Natalina s.s.* and *Afrorhytida*, only two characters are shared between the two: left mantle lobes separate with the lower one expanded only

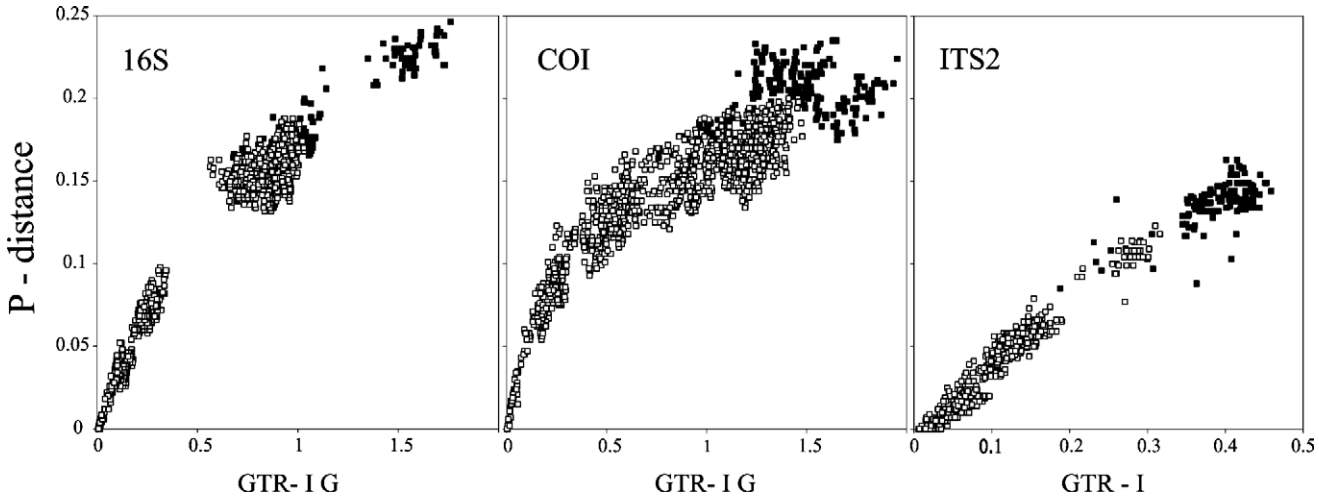

Fig. 4. Plots of observed pairwise differences (P-distance) versus estimated divergences for 16S (GTR − I Γ), COI (GTR − I Γ) and ITS2 (GTR − I), respectively. The open square symbol represent divergences among taxa within *Natalina s.s.*, while filled square symbol represents divergence between the ingroup and the outgroup.

on the right (4) and the relatively long epiphallus (18) (0.5–0.75 × length of penis). For the former character, however, an exception is the autapomorphy of a hypertrophied mantle edge in *N. wesseliana* (*Natalina s.s.*), and in the latter character, the relatively long epiphallus state is shared with an outgroup species, *Nata tarachodes* (though in reality these are probably not homologous states). There are three cases where the morphology is incongruent with the molecular data in terms of the phylogenetic relationship among the three subgenera. Firstly, both *Capitina* and *Afrorhytida* have the penis to the left of the optic retractor (15), while *Natalina s.s.* shares the converse state with the outgroup taxa, *Nata vernicosa* and *N. tarachodes*. Secondly, the bursa duct lying beside rather than within in the convolutions of the sperm-oviduct (21) is a character shared by *Capitina* and *Afrorhytida*. Notwithstanding this phylogenetic incongruence, however, the penis being right of the optic retractor and the bursa duct located within the convolutions of the sperm-oviduct are ingroup synapomorphies consistent with the monophyly of *Natalina s.s.* Thirdly, the connection of the penis to the epiphallus by a tissue web (17) is common to both *Natalina s.s.* and *Capitina*. Once again, aside this incongruence regarding the sister relationship among subgenera, the penis being free of the epiphallus represents a synapomorphy defining *Afrorhytida*.

Concerning the radula, the latero-marginal transition (10) and the number of lateral teeth (11) are the most phylogenetically consistent with the molecular data, although not without exception. Two evolutionary trajectories in radula morphology are evident in *Natalina s.l.* Firstly, there is a reduction in the size of the outer lateral teeth, resulting in a gradual transition between the laterals and marginals as an autapomorphy defining *Capitina*. Secondly, in conjunction with a reduction in the number of lateral teeth (11), there is an abrupt differentiation (no intermediary teeth) between the laterals and marginals in *Natalina s.s.* Little phylogenetic congruence is evident, however, for either the number of marginal teeth (12) or the ratio of laterals to marginals, with both exhibiting overlapping variability within *Natalina s.s.* and *Afrorhytida*. Nevertheless, these and other radula characters are useful as diagnostic characters for species-level taxonomic purposes; for instance, in separating the *N. compacta–N. eumacta* clade from the remaining members of the *cafra*-group, and in differentiating the four *Afrorhytida* taxa. The remaining two characters, namely, the highly textured skin (5) in *N. wesseliana* and the spathulate shape of inner lateral teeth (14) in *A. trimeni*, are autapomorphies and accordingly are not phylogenetically informative.

4. Discussion

The molecular data provide a highly resolved phylogeny which confirms *Natalina s.l.* as a monophyletic radiation and delineates three strongly supported subclades which are consistent with Watson's (1934) conception of the taxa (subgenera) *Capitina*, *Afrorhytida* and *Natalina s.s.* Sequence divergences between the three subgenera are exceptionally high, particularly under a corrected model of sequence evolution. While mutation rate estimates vary considerably among terrestrial molluscs, ranging from a typical rate of 1–3% per million years (m.y.) (e.g. Hugall et al., 2003; Spencer et al., 2006) to as great as 10% per m.y. (Chiba, 1999; Hayashi and Chiba, 2000; Thacker and Hadfield, 2000), a preliminary estimate for rhytidids provided by Spencer et al. (2006) based on the COI phylogeny of the New Zealand endemic subfamily Paryphantinae is most relevant to the current study. Based on the COI dataset and the same method of analysis (neighbour-joining) and model of sequence evolution (GTR), estimated mean sequence divergence between the three subgenera considered here (maximum of 25%, between the 'Transkei–East London' clade within the *cafra*-group and *A. knysnaensis*) is considerably greater than that separating the four genera within the Paryphantinae (approximately 8%). Indeed, divergences between the major lineages within *Natalina s.l.* are comparable to those between genera across the two New Zealand subfamilies (Paryphantinae and Rhytidinae). Considering a range of mutation rates (0.7–2.4% per m.y.), Spencer et al. (2006) concluded that a relatively slow mutation rate (<1% pairwise/m.y.) was most consistent with the geological evidence, namely the separation of the Three King Islands from the Mainland approximately 10–15 m.y.a., and the estimated divergence of the endemic genus *Rhytidarex* from remaining Paryphantinae genera. Based on this mutation rate, the initial diversification within *Natalina s.l.* would have occurred early in the Miocene epoch. Without a fossil-calibrated phylogenetic estimate of mutation rate, however, and considering that the Spencer et al. (2006) estimate is in the lower range reported for molluscs (but see Douris et al., 1998; Marko, 2002), such inference needs to be treated cautiously. Nevertheless, based on the best evidence available for the family Rhytididae, cladogenesis of the three major lineages most probably predates the Pliocene and is possibly associated with major drying, contraction and fragmentation of mesic habitat in sub-Saharan Africa commencing in the mid-Miocene (Dingle and Hendey, 1984; Jacobs, 2004; Linder, 2003; Scott et al., 1997).

Concordant with the molecular phylogeny, the morphological dataset provides strong support for the monophyly of *Natalina s.l.*, confirming the primary dichotomy within southern African rhytidids identified by Watson (1934) and his erection of *Nata* as an entity distinct from *Natalina*. Although there were conflicting signals among the morphological characters regarding the sister relationships between *Natalina* and *Afrorhytida*, as identified by the molecular analysis, several character states are consistent with the monophyly of *Natalina s.s.* and *Afrorhytida.*, while three autapomorphies clearly differentiated *Capitina*, thus providing a strong basis for the recognition of three natural groupings within *Natalina s.l.* Based on these data, in conjunction with the strong support from the molecular data for Watson's classification and the relatively deep molecular divergences, we propose that the three subgenera be elevated to generic status. In the following discussion we examine each of the three genera in turn, providing qualification of the proposed ranking and detailing key morphological characters of diagnostic importance.

4.1. Capitina Watson, 1934

When proposing the subgenus *Capitina* for *Natalina schaerfiae*, Watson (1934) was clearly of the opinion that it represented a highly distinctive taxon. Differentiation of *Capitina* from the two other genera is supported by a number of major morphological autapomorphies, namely the merging of the left body-lobes of the mantle, the very short epiphallus and the lack of differentiation between the marginal and lateral teeth of the radula (Fig. 3). Importantly, the degree of divergence in the radula alone is comparable to that found between genera in both Australia and New Zealand (see Connolly, 1939; Powell, 1930; Smith, 1987). In terms of molecular data as well, this taxon is clearly distinct within the *Natalina s.l.* radiation, being exceptionally divergent from all remaining species based on both the mitochondrial and nuclear data. Within *Capitina*, two lineages are evident, namely *C. schaerfiae* which is primarily restricted Afrotemperate forest habitats at somewhat higher elevations on the southern slopes of the Riviersonderendberge, and an un-named lineage restricted primarily to coastal fynbos between Hermanus and Cape Agulhas (Herbert and Moussalli, unpublished data). While the molecular data suggest relatively recent divergence, specimens from the coastal fynbos are clearly morphologically distinct from typical *C. schaerfiae* specimens from Afrotemperate forest, a fact noted also by Watson (1934). The former differ consistently in having a notably paler and more elevated shell, a smaller embryonic shell (4.0–5.3 mm versus 6–7 mm), fewer teeth in the radula (formula approximately 20 + 1 + 20 versus 30 + 1 + 30) and are generally much more strongly banded, albeit polymorphically so. On account of these differences, we propose that these lineages be recognised as separate subspecies. Both are of conservation concern, being narrow-range endemics that are confined to vegetation units of limited extent – isolated western fragments of southern Afrotemperate forest in the case of *C. schaerfiae schaerfiae*, and western Overberg dune strandveld and Agulhas limestone fynbos (*sensu* Mucina and Rutherford, 2006) in the case of the un-named coastal subspecies.

4.2. Natalina Pilsbry, 1893

The monophyly of *Natalina s.s.* is strongly supported by both the morphological and molecular data. When compared to *Afrorhytida* and *Capitina*, members of the genus exhibit a number of morphological synapomorphies which render it readily diagnosable. The three most notable characters are the abrupt transition from lateral to marginal teeth (no intermediaries), a well developed oviduct caecum, and duct of bursa copulatrix positioned along the central axis of the sperm oviduct (see Fig. 3). Within the genus there are three highly divergent monophyletic clades, with *N. wesseliana* being the most morphologically distinct owing to its large size and very coarse skin texture, the genital pore lying dorso-laterally and well posterior to the right optic tentacle and the mantle edge hypertrophied with the left body lobes confluent. In addition to its morphological distinctiveness, *N. wesseliana* is the northernmost species of *Natalina s.l.*, restricted to coastal and scarp forest remnants north of a recognised biogeographic barrier, the Mfolozi River (Cooper, 1985). The species is currently listed as vulnerable on the IUCN Red List of Threatened Species due to ongoing habitat loss and degradation (IUCN, 2007).

The second lineage within *Natalina s.s.*, the *cafra*-group, contains most of the large and familiar cannibal snails of eastern South Africa which have until now been identified with four nominate taxa, namely *N. beyrichi* (Martens, 1890), *N. cafra* (Férussac, 1821), *N. compacta* Connolly, 1939 and *N. eumacta* (Melvill and Ponsonby, 1892). Of these, *N. beyrichi* is easily identified on conchological grounds on account of its characteristically depressed shell and wide umbilicus, but discrimination between the three remaining species has often proved difficult due to overlap in their respective shell proportions (height:diameter) and colour. In terms of the anatomical characters considered here, all four nominal taxa exhibit almost identical states. Individuals identified conchologically as *N. beyrichi* from coastal localities in the Transkei region, however, consistently cluster in a well-supported clade in our analyses of molecular data, confirming that this is a distinct species with limited distribution. This is further reinforced by the fact that *N. beyrichi* is partially sympatric with *N. compacta*-like representatives of the 'Transkei–East London' clade (see Fig. 1), which consistently possess a much more globose shell. At a finer scale, these two taxa occur syntopically within scarp forest remnants in the Dwesa-Haven area, E. Cape. That they very clearly retain their separate identities in this area of overlap, both molecularly and morphologically, strongly suggests reproductive isolation and completed speciation. Additional, faster evolving nuclear markers are desirable, however, to fully qualify this observation.

In regard to the remaining nominal species within the *cafra*-group, there is clear incongruence between the molecular data and current taxonomy, necessitating taxonomic revision. Firstly, the situation with regard to *N. cafra s.l.* is complicated by the evident paraphyly, where the split between *N. cafra* (KZN) and all remaining representatives of the *cafra*-group (including *N. beyrichi*) is basal and well supported. Secondly, our results demonstrate that topotypic material of *N. compacta* (Mountain Drive, see Fig. 3) belongs within the same clade as topotypic material of *N. cafra* (e.g. Coerney), indicating that *N. compacta* is based on nothing more than globose individuals of the typical E. Cape *N. cafra*. The name *N. compacta* must therefore be considered a synonym of *N. cafra s.s.* and cannot be applied to the globose *compacta*-like specimens occurring in either the Amathole or the Transkei–East London region. Lastly, the well supported 'Transkei–East London' clade represents a mixture of specimens designated as either *N. eumacta* or *N. compacta* based on shell size, shape and pigmentation. Despite the conchological variability associated with this clade, there does exist one morphologically diagnostic character, namely the possession of eight pairs of lateral teeth per radula row instead of the five to six in all other lineages within the *cafra*-group including *N. beyrichi* (Herbert and Moussalli, unpublished data).

Evidently, there is lack of congruence between shell form and the molecular data within the *cafra*-group. As noted by Watson (1934: 157, 158) relative shell proportions are frequently extremely variable within species of *Natalina s.l.* and are often of little taxonomic value (*N. beyrichi* being a notable exception). Given our results, we recognise only two species with the *cafra*-group, *N. beyrichi* and *N. cafra*. Within *N. cafra* there are four geographically oriented, reciprocally monophyletic subclades and we recog-

nise each of these as an evolutionary significant unit (ESU) (de Guia and Saitoh, 2007; Moritz, 1994). Specifically, we propose an alternative taxonomy for the *cafra-compacta-eumacta* complex, one which recognises these genetically distinct lineages as subspecies. Shell shape in the *N. cafra cafra* subspecies (south-western E. Cape) is the most variable and includes the typical, somewhat depressed *cafra* form as well as both even more depressed individuals and the globose *compacta* form. In both the coastal Transkei–East London and inland Amathole populations, the shell is consistently highly globose. While the name *eumacta* is available for the former, a new name is needed for the latter and we provisionally refer to it as *N. cafra* subsp. n. (Amathole). Lastly, although the distinctiveness of *N. cafra* (KZN) is well supported by both the mitochondrial and the nuclear data, the lack of morphological differentiation between this clade and members of *N. cafra cafra*, in conjunction with the paraphyly being principally based on the mitochondrial dataset, hinders specific recognition. We therefore provisionally refer to this northern population as *N. cafra* subsp. n. (KZN). It is somewhat less variable in shell proportions than *N. cafra cafra*, the majority of specimens being of intermediate proportions. As was the case with Climo's (1978) rationalisation of the New Zealand rhytidid, *Powelliphanta hochstetteri*, which led to the reclassification of *P. superba*, *P. lingaria*, *P. rossiana* and *P. fiordlandica* as subspecies thereof, incipient speciation associated with vicariant events during Quaternary climate fluctuations in conjunction with limited introgression and hybridisation perhaps underpins the various geographic forms observed within the *cafra*-group. In the same light, the subspecific ranking which we propose here is done primarily to facilitate further study of this complex, preferably with greater geographic sampling and attention to morphometrics (e.g. Kameda et al., 2007; Walker et al., 2008), and the application of faster evolving nuclear markers. Of particular interest would be a comparison of environmental versus genetic correlates of both local and regional variation in morphology, to assess the relative roles of historical processes and selection in the diversification of this group (Davison, 2002; Fiorentino et al., 2008).

The third lineage within *Natalina s.s.* is representative of a number of relatively small species occurring only in forest habitats (principally in Afrotemperate and mistbelt forest) in KwaZulu-Natal and Mpumalanga, namely *N. quekettiana*, *N. inhluzana*, and *N. reenenensis*. As rainforest specialists, the biogeography of this group is a reflection of the highly fragmented distribution of this forest in south-eastern Africa (Cooper, 1985; Low and Rebelo, 1996; Midgley et al., 1997). Accordingly, the type localities for these three nominal species are restricted to individual fragments of this rainforest archipelago with most remnants being small (<1 km^2). As indicated in the methods, with the exception of *N. quekettiana*, these three nominal species are known only from empty shells, despite recent targeted surveys. Nevertheless, several living specimens belonging to the *N. quekettiana* complex from other forests in the same broader region have been found. In terms of the morphological data considered here, all the specimens within this lineage exhibit identical character states. There exists some geographically oriented variability, however, in shell size and colouration, and in radula dentition. For instance, northern populations (Ngome and Entumeni) are consistently larger at adult size and the radula has only six pairs of lateral teeth per transverse row, compared to 8–9 in the other lineages (Herbert and Moussalli, unpublished data). In the case of the Ngome population, they also have a distinctively dark brown shell and bright yellow pigmentation on the mantle edge and pedal margin. Conversely, specimens obtained from the Drakensberg are not clearly distinguishable from topotypic *N. quekettiana* from Ferncliffe, Pietermaritzburg, exhibiting much overlap in shell dimensions and colour. Moreover, two distinct haplotype clusters are found within the Drakensberg, one closely and one distantly related to *N. quekettiana s.s.*, with molecular divergence comparable to those within the *cafra*-group. Owing to the difficulty in obtaining sufficient representatives of this complex, taxonomic resolution must await a larger and more spatially representative molecular dataset coupled with detailed morphometrics.

4.3. Afrorhytida Möllendorff, 1903

Of the four well-supported clades identified within *Afrorhytida* based on the molecular data, three are broadly consistent with current taxonomy, namely *A. kraussi*, *A. knysnaensis* and the section *Hyperrhytida*, while the fourth represents an undescribed lineage restricted to the Kei and Mbashe River catchments (see Fig. 1). Based on the morphological data presented here, there are only two synapomorphies that clearly unite species of *Afrorhytida*, namely the epiphallus and lower vas deferens not being joined laterally to the penis by a connective tissue web and the radula having marginal teeth of intermediate size between the large outer laterals and the otherwise largely vestigial outer marginals.

Populations referable to the *A. kraussi* complex are found widely in both forest and mesic fynbos habitats in the southern Cape (primarily the Cape Fold Mountains), from the western Langeberge east to Van Stadensberge near Port Elizabeth (Fig. 1). These form a well supported and divergent clade with strong internal geographic structure. For the most part, we can currently define no features which might allow these subclades to be reliably diagnosed morphologically. An exception is the De Hoop population, specimens of which are easily distinguishable in shell form and colour from others within the *kraussi* complex (see Fig. 3), being smaller in size, thicker-shelled, notably paler in shell pigmentation (almost white) and the apex, including embryonic shell, having distinct maroon-brown pigmentation. These albeit qualitative characters and the restriction of this form to coastal limestone fynbos (Fig. 1), are partly convergent with those differences observed between the two subspecies of *Capitina schaerfiae*. Representatives from the western forest isolates are also distinctive, though to a lesser extent, in that they attain greater size and exhibit a distinctively redder body pigmentation. Given the information currently available, however, we consider this clade to be an unresolved complex that requires additional sampling and further study. This should be prioritised because both the De Hoop and the western Afrotemperate forest populations are of high conservation concern due to their very limited distributions.

The second clade within *Afrorhytida* represents a complex of nominate taxa which we have been unable to discriminate morphologically, namely *N. knysnaensis*, *N. coerneyensis* and *N. insignis*. Since *N. knysnaensis* (Pfeiffer, 1845) is the earliest of the three available names, we refer to it as the *A. knysnaensis* complex. This taxon, centred on the catchments of the Great Fish and Sundays rivers (Fig. 1), clusters as the sister taxon to the *N. kraussi* group and like that complex shows considerable internal genetic diversity. In contrast to all complexes considered so far, there exists little obvious geographic structuring, a pattern consistent with the fact that the complex occurs essentially within a single, more or less continuous biome, the Albany Thicket (Mucina and Rutherford, 2006). Accordingly, the high phylogenetic diversity within this clade can perhaps be explained as the persistence of a single large and/ or several well connected populations throughout most of the Quaternary and the retention of ancestral polymorphism. Qualification of this hypothesis, however, would require examination of additional markers, preferably with mutation rates comparable to those found in mitochondrial DNA. The *A. knysnaensis* complex is differentiated from the *A. kraussi* complex by having a more globose shell, a more gradual latero-marginal transition in the radula, and in possessing an oviduct caecum, albeit small.

In regards to the third clade within *Afrorhytida*, Watson (1934) designated the sister taxa *N. trimeni* and *N. arguta* to a separate section, differing from both *A. kraussi* and *A. knysnaensis* in the width of the umbilicus, the relative strength of radial shell sculpture and in several aspects of radula dentition (see also Connolly, 1939). Topotypic material representing *N. arguta*, however, shows limited molecular divergence from *N. trimeni* and the taxa are identical in terms of the morphological characters examined here. Since the supposedly diagnostic shell characters relate only to a difference in size and slight differences in umbilical width and strength of sculpture, all of which could easily be encompassed within the variability of a single species, we consider them to be conspecific and employ the earlier of the two names, *A. trimeni*. The characters given for *Hyperrhytida* thus relate only to species-specific features of *A. trimeni* and *Hyperrhytida* does not therefore represent a supraspecific entity diagnosable in terms of synapomorphic characters. Like *A. kraussi*, *A. trimeni* has a rather more depressed shell form (see Fig. 3) and exhibits a strong preference for mesic habitat, specifically rainforest fragments between East London and Port Elizabeth. Due to the limited and highly fragmented distribution of forests in this area, the species should receive high conservation status and effort to ensure adequate representation of coastal rainforest within the regional reserve system.

Lastly, our molecular data identifies a fourth currently undescribed lineage of *Afrorhytida* for specimens obtained from recent survey work. This undescribed taxon closely resembles globose specimens of *A. knysnaensis* in shell shape, but differs consistently in its radula morphology, having many fewer marginal teeth (<10 versus >20), a feature that is shared with *A. trimeni*. While the lineage is clearly distinct based on both the mitochondrial and nuclear (ITS2 + structural analysis) data, the molecular data provide no resolution regarding its phylogenetic placement within *Afrorhytida*. Further, like *A. knysnaensis* and *A. kraussi*, this taxon exhibits a considerable degree of phylogenetic diversity, with 58% (COI, GTRIG) separating the Cathcart and Wedgeley populations. Whether any morphological differentiation underlies this diversity, however, is uncertain as the single specimen from Cathcart was juvenile. The Cathcart specimen was found at a relatively high elevation >1200 m, in close proximity to the Windvogelberg Massif, while the remaining two specimens from Elizweni and Wedgeley were found at lower elevations (<850 m). Surveys of the upper catchment of the Kei River are ongoing, and we anticipate that additional material will facilitate clarification of the taxonomic status of this Cathcart population.

4.4. Conclusion

Overall, this study presents a well-resolved molecular phylogeny, which on the one hand, strongly supports Watson's supra-specific classification, and on the other, clearly indicates the need for taxonomic revision. Additional fine scale molecular and morphological studies are needed to elucidate the evolutionary processes underpinning the substantial genetic diversity within the four principal species complexes identified in this study, namely the *cafra*-group, and the *N. quekettiana*, *A. kraussi* and *A. knysnaensis* complexes. At the broader scale, biogeographic patterns identified here are generally concordant with known centres of endemicity. For instance the Cathedral Peak region harbours two narrowly endemic achatinid species (*Cochlitoma omissa* Bruggen, 1965 and *C. montistempli* Bruggen, 1965), while the distribution of a related, possibly sister species, *C. dimidiata* (Smith, 1878), is broader, including Afrotemperate and mistbelt forest habitat in KwaZulu-Natal and Mpumalanga (Herbert and Kilburn, 2004). This pattern is similar to that in the *N. quekettiana* complex, in which there are two highly divergent lineages in the Cathedral Peak–Injasuthi region, and two other lineages occurring more broadly in mistbelt and scarp forests of KwaZulu-Natal (and possibly into Mpumalanga). In terms of the *cafra*-group, Pondoland is a recognised major regional centre of botanical diversity and endemicity (van Wyk and Smith, 2001), a pattern also reflected in terrestrial molluscs with many endemics restricted to this region in addition to *N. beyrichi* (Bursey and Herbert, 2004; Herbert, 2006; Herbert and Kilburn, 2004). Similarly, biogeographic structure observed within *Afrorhytida*, is strongly concordant with the radiation of tail-wagger snails, subgenus *Sheldonia s.s.* (family Urocyclidae) (Moussalli and Hebert, unpublished). Given the preliminary insights presented here and their generally high diversity in southern Africa combined with high levels of local endemism (Govender, 2008), we suggest that terrestrial molluscs provide an ideal system for investigating the historical biogeography of this region.

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Appendix A

Specimens used in molecular analysis

Species	Locality	NMSA	COI	16S	ITS2
Nata tarachodes (Connolly, 1912)	Spes Bona Bush, Kalk Bay	W2471	FJ262172	FJ262238	FJ262304
Nata vernicosa (Krauss, 1848)	Ngoye Forest, near Eshowe	W3321	FJ262173	FJ262239	FJ262305
Natalina beyrichi (Martens, 1890)	Haven	W4149	FJ262175	FJ262241	FJ262307
Natalina beyrichi (Martens, 1890)	Hluleka N.R.	W2895	FJ262174	FJ262240	FJ262306
Natalina beyrichi (Martens, 1890)	Mbotyi	W4123	FJ262176	FJ262242	FJ262308
Natalina beyrichi (Martens, 1890)	Mkambati N.R.	V9220	FJ262177	FJ262243	FJ262309
Natalina beyrichi (Martens, 1890)	Notintsila	W4734	FJ262178	FJ262244	FJ262311

Appendix A. (*continued*)

Species	Locality	NMSA	COI	16S	ITS2
Natalina beyrichi (Martens, 1890)	Port St. Johns	W4125	FJ262191	FJ262257	–
Natalina cafra (Férussac, 1821) (KZN)	Nkandla Forest	W1167	FJ262179	FJ262245	FJ262310
Natalina cafra (Férussac, 1821) (KZN)	Eshowe (Dlinza Forest)	V8087	FJ262180	FJ262246	FJ262312
Natalina cafra (Férussac, 1821) (KZN)	Tugela Mouth	W4699	FJ262192	FJ262258	–
Natalina cafra (Férussac, 1821) (KZN)	Karkloof Forest	W5144	FJ262184	FJ262250	FJ262315
Natalina cafra (Férussac, 1821) (KZN)	Mtamvuna Gorge	W308	FJ262188	FJ262254	–
Natalina cafra (Férussac, 1821) (KZN)	Durban	W5025	FJ262187	FJ262253	–
Natalina cafra (Férussac, 1821) (KZN)	Bulwer	W1417	FJ262185	FJ262251	FJ262316
Natalina cafra (Férussac, 1821) *s.s.* (SW E. Cape)	Coerney	W4220	FJ262183	FJ262249	FJ262314
Natalina cafra (Férussac, 1821) *s.s.* (SW E. Cape)	Baviaanskloof	W3255	FJ262181	FJ262247	FJ262313
Natalina cafra (Férussac, 1821) *s.s.* (SW E. Cape)	Jeffreys Bay	W4225	FJ262182	FJ262248	FJ262318
Natalina compacta Connolly, 1939 *s.s.* (E. Cape)	Grahamstown, Mountain Drive	W5212	FJ262190	FJ262256	FJ262320
Natalina compacta Connolly, 1939 (Amathole)	Hogsback	W4193	FJ262186	FJ262252	FJ262317
Natalina compacta Connolly, 1939 (Amathole)	Hogsback	V9792	FJ262189	FJ262255	FJ262319
Natalina compacta Connolly, 1939 ('Transkei')	East London	W852	FJ262195	FJ262261	FJ262323
Natalina compacta Connolly, 1939 ('Transkei')	Haven	W4150	FJ262196	FJ262262	FJ262324
Natalina compacta Connolly, 1939 ('Transkei')	Manubi Forest	W4160	FJ262197	FJ262263	FJ262325
Natalina eumacta (M and P, 1892)	Mbashe River at N2	W4627	FJ262193	FJ262259	FJ262321
Natalina eumacta (M and P, 1892)	Collywobbles	W4077	FJ262194	FJ262260	FJ262322
Natalina eumacta (M and P, 1892)	Nxaxo	W2721	FJ262199	FJ262265	FJ262327
Natalina eumacta (M and P, 1892)	Mazeppa Bay	W4161	FJ262198	FJ262264	FJ262326
Natalina quekettiana (M and P, 1893) complex	Ferncliffe, Pietermaritzburg	W4262	FJ262200	FJ262266	FJ262328
Natalina quekettiana (M and P, 1893) complex	Bulwer	W1490	FJ262205	FJ262271	FJ262333
Natalina quekettiana (M and P, 1893) complex	Cathedral Peak	W4596	FJ262207	FJ262273	FJ262336
Natalina quekettiana (M and P, 1893) complex	Oqalweni, Cathedral Peak	W4084	FJ262201	FJ262267	FJ262329
Natalina quekettiana (M and P, 1893) complex	Injasuthi	V7992	FJ262202	FJ262268	FJ262330
Natalina quekettiana (M and P, 1893) complex	Monks Cowl	W4101	FJ262208	FJ262274	FJ262337
Natalina quekettiana (M and P, 1893) complex	Ngome Forest	W3305	FJ262203	FJ262269	FJ262331
Natalina quekettiana (M and P, 1893) complex	Entumeni Forest, Eshowe	W3310	FJ262209	FJ262275	FJ262332
Natalina wesseliana (Kobelt, 1876)	Hluhluwe G.R.	V7668	FJ262210	FJ262276	FJ262338
Natalina wesseliana (Kobelt, 1876)	Xai-Xai, Mozambique	L7364	FJ262212	FJ262278	FJ262340
Natalina wesseliana (Kobelt, 1876)	Cape Vidal	V7980	FJ262211	FJ262277	FJ262339
Natalina wesseliana (Kobelt, 1876)	Hlatikulu, near Jozini	W4831	FJ262213	FJ262279	FJ262341
Natalina (Afrorhytida) knysnaensis (Pfeiffer, 1846)	Fort Beaufort	W5211	FJ262217	FJ262283	FJ262349
Natalina (Afrorhytida) knysnaensis (Pfeiffer, 1846)	Cradock	W4248	FJ262214	FJ262280	FJ262342
Natalina (Afrorhytida) knysnaensis (Pfeiffer, 1846)	Double Drift G.R.	W4749	FJ262218	FJ262284	FJ262350
Natalina (Afrorhytida) knysnaensis (Pfeiffer, 1846)	Somerset East	W4846	FJ262215	FJ262281	FJ262343
Natalina (Afrorhytida) knysnaensis (Pfeiffer, 1846)	Wapadskloof, Bedford area	W5255	FJ262216	FJ262282	FJ262344
Natalina (Afrorhytida) knysnaensis (Pfeiffer, 1846)	Koedoeskop, Somerset East	V6774	FJ262219	FJ262285	FJ262345
Natalina (Afrorhytida) sp. n.	Cathcart	W4844	FJ262220	FJ262286	FJ262346
Natalina (Afrorhytida) sp. n.	Elizweni, East London	W5252	FJ262221	FJ262287	FJ262347
Natalina (Afrorhytida) sp. n.	Wedgeley, Kei River Valley	W5228	FJ262222	FJ262288	FJ262348
Natalina (Afrorhytida) kraussi (Pfeiffer, 1846)	Attakwas Mts	W4780	FJ262226	FJ262292	FJ262354
Natalina (Afrorhytida) kraussi (Pfeiffer, 1846)	Diepwalle Forest, Knysna	W4845	FJ262228	FJ262294	FJ262356
Natalina (Afrorhytida) kraussi (Pfeiffer, 1846)	Touwsrivier, Wilderness	W4784	FJ262229	FJ262295	FJ262357
Natalina (Afrorhytida) kraussi (Pfeiffer, 1846)	Tradoupas, Swellendam area	W4678	FJ262230	FJ262296	FJ262358
Natalina (Afrorhytida) kraussi (Pfeiffer, 1846)	Featherbed N.R., Knysna	W4671	FJ262227	FJ262293	FJ262355
Natalina (Afrorhytida) kraussi (Pfeiffer, 1846)	Grootvadersbosch N.R.	W3350	FJ262231	FJ262297	FJ262359
Natalina (Afrorhytida) kraussi (Pfeiffer, 1846)	Outeniqua Mts	W3211	FJ262232	FJ262298	FJ262360
Natalina (Afrorhytida) kraussi (Pfeiffer, 1846)	Cloetesberge	W3207	FJ262233	FJ262299	FJ262361
Natalina (Afrorhytida) kraussi (Pfeiffer, 1846)	De Hoop N.R.	W3297	FJ262234	FJ262300	FJ262362
Natalina (Afrorhytida) trimeni (M and P, 1892)	Hamburg	W5254	FJ262223	FJ262289	FJ262351
Natalina (Afrorhytida) trimeni (M and P, 1892)	Kap River N.R.	W4206	FJ262224	FJ262290	FJ262352
Natalina (Afrorhytida) arguta M and P, 1907	Umtiza N.R., East London	W4182	FJ262225	FJ262291	FJ262353
Natalina (Capitina) schaerfiae subsp. n.	Cape Agulhas	W3360	FJ262236	FJ262302	FJ262364
Natalina (Capitina) schaerfiae subsp. n.	Gansbaai	W3201	FJ262235	FJ262301	FJ262363
Natalina (Capitina) schaerfiae (Pfeiffer, 1861)	Oubos, Riviersonderend	W5672	FJ262237	FJ262303	FJ262365

Appendix B

Description of morphological characters

In his discussion of the relationships of South African rhytidids, Watson (1934) laid the foundation for the study of rhytidid morphology. This work has been the primary resource informing our investigation of morphology, but all characters have been re assessed through direct observation and gaps in the available data filled where ever possible. The definition of characters and their coding was guided by the principles outlined by Sikes et al. (2006). Inapplicable characters were coded as ? = missing.

Shell characters

1. Embryonic shell diameter. Clear differences between taxa in terms of adult size are evident, but these can be difficult to assess in specimens that are not adult. However, these differences are also reflected in the size of the embryonic shell, the limit of which is evident in the majority of specimens. Its maximum visible diameter in apical view can be divided into distinct size classes. Character coding: 0 = <2.5 mm, 1 = 2.5–7 mm, 2– >7 mm.
2. Embryonic shell sculpture. This is a distinctive character separating *Nata* with a smooth and glossy embryonic shell, and *Natalina* with a lusterless, axially ribbed embryonic shell. Character coding: 0 = smooth and glossy, 1 = sculptured with axial ribs, lusterless.
3. Peristome. The outer lip of the shell is generally thin and membranous and growth thus indeterminate, but in some taxa growth is evidently determinate, adult size being marked by a distinct, albeit slight, thickening of the outer lip. This may be accompanied also by a down turn of the last portion of the body whorl just prior to the aperture and a slight flattening or indentation on the outer lip below it insertion at the suture. Character coding: 0 = outer lip of adult thin and membranous, 1 = outer lip of adult somewhat thickened.

External anatomy characters

4. Left body lobe of mantle. The body lobe on the mantle edge to the left of the pneumostome is developed to varying degrees, ranging from two small, widely spaced lobes (upper and lower) to one continuous skirt-like lobe extending over the neck to the left of the pneumostome. Character coding: 0 = lower left body lobe expanded on both sides, 1 = lower left body lobe expanded only on its right, 2 = mantle edge hypertrophied with left body-lobes confluent, their junction being marked only by a sinuous disjunction between the smaller upper lobe and the much wider lower lobe, 3 = left body lobe forms one continuous lobe.
5. Skin texture (particularly in neck region). Generally, the texture of the skin is somewhat variable, but difficult to describe in categorical terms. In most species it is finely to moderately granular, however, in *Natalina wesseliana* the skin, particularly in the neck region, is very coarsely textured with pitted granules dorsally. Character coding: 0 = fine to moderate, 1 = coarse.
6. Labial palps. The postero-lateral region of the oral disc, below the lower tentacles is frequently expanded laterally to form well developed labial palps (oral lobes). Character coding: 0 = labial palps absent, 1 = labial palps present.
7. Genital pore. The position of the genital pore on the right side of the neck is variable. Character coding: 0 = ventral to pneumostome, 1 = ventral and just posterior to optic tentacle, 2 = dorsolaterally on neck, well posterior to optic tentacle.

Radula characters

8. Radula – number of complete transverse rows. The number of rows of radula teeth varies from 24 to 80. Although there may be some intraspecific variation related to age/size, if mature specimens only are considered, this character is relatively clearly resolved into two groups, respectively, with fewer or greater than 40 rows. Character coding: 0 = <40 rows, 1 = >40 rows.
9. Rachidian tooth. A rachidian tooth may or may not be present, even when present it is invariably small. Character coding: 0 = absent, 1 = present.
10. Latero-marginal transition: the transition from lateral to marginal teeth may be abrupt with the outermost lateral tooth dwarfing the innermost marginal, or there may be a small number of teeth of intermediate size (2–5), a larger number of intermediary teeth (>5), or the transition may be gradual, such that there is no clear distinction between the series. Character coding: 0 = 2–4 intermediaries, 1 = >5 intermediaries , 2 = abrupt. 3 = gradual.
11. Number of lateral teeth. Not applicable in species where there is no clear distinction between lateral and marginal series. Character coding: 0 = <10, 1 = >10.
12. Number of marginal teeth. Not applicable in species where there is no clear distinction between lateral and marginal series. Character coding: 0 = 4 or less, 1 = 20–30, 2 = 5–9, 3 = 10–20.
13. Ratio of number of lateral teeth: number of marginal teeth. Not applicable in species where there is no clear distinction between lateral and marginal series. Character coding: 0 = >1.2, 1 = <0.25, 2 = 0.30–0.7, 3 = ±1.0 (0.8–1.2).
14. Shape of inner lateral teeth. This character refers to an autapomorphic character state in *Afrorhytida trimeni*. In *Nata dumeticola* most of the radula teeth are also broad and flat, but this does not apply to the inner laterals and the condition is not homologous with that seen in *A. trimeni*. Character coding: 0 = aculeate, 1 = spathulate.

Genital tract characters

15. Penis position in relation to right optic retractor. The penis may lie either the right of the of the right optic retractor muscle, or to its left, with the muscle passing between the penis-epiphallus and the vagina-sperm-oviduct. Character coding: 0 = penis to right of optic retractor, 1 = penis to left of optic retractor.
16. Penis shape. In *Natalina s.l.*, the penis is long and cylindrical, whereas in *Nata* it may be variously shaped, but is never long and cylindrical. Character coding: 0 = penis not elongate cylindrical, 1 = penis elongate cylindrical.
17. Penis/epiphallus/lower vas deferens. In *Nata*, the epiphallus (if present) is fused to the side of the penis, but in *Natalina s.l.* the two are not closely conjoined laterally and the epiphallus (and lower vas deferens) may either be connected to the penis by a web of connective tissue or may be completely free of the penis. Character coding: 0 = epiphallus fused to side of penis, 1 = epiphallus and lower vas deferens separate from penis but joined to it by connective tissue web, 2 = epiphallus and lower vas deferens free of penis.
18. Epiphallus length. Character coding: 0 = very short <0.1 length of penis, 1 = short <0.5 length of penis, 2 = long, 0.5–0.75 length of penis.
19. Vagina length. In *Nata* the vagina is very short, whereas in *Natalina s.l.* it is considerably longer. Character coding: 0 = short, 1 = long.

20. Duct of bursa copulatrix: length and breadth. In *Nata* the duct of the bursa copulatrix is relatively short and often broader in the basal region, whereas in *Natalina s.l.* it is very much longer and more slender. Character coding: 0 = relatively short (shorter than sperm-oviduct) and basally swollen, 1 = long (longer than sperm-oviduct) and more or less uniformly narrow.
21. Duct of bursa copulatrix: position. The bursa duct may either lie alongside the sperm-oviduct or lie along its central axis, deep within its convolutions. Character coding: 0 = bursa duct beside sperm-oviduct, 1 = bursa duct within convolutions of sperm-oviduct.
22. sperm-oviduct. The sperm-oviduct may be straight or slightly sinuous, with only superficial folds in the oviducal portion, or it may be convoluted with strong, sinuous folds about a central axis. Character coding: 0 = sperm-oviduct more or less straight, 1 = sperm-oviduct convoluted.
23. Oviduct caecum. A cylindrical caecum may arise from the oviduct, close to its junction with the vagina. Character coding: 0 = caecum absent, 1 = caecum present, well developed, 2 = caecum present, but small.

Appendix C. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ympev.2009.02.018.

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