From depressed to detached: extreme shell shape variation in some Peruvian *Bostryx* species (Gastropoda: Bulimulidae)

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Abstract: This paper investigates *Bostryx* species from Peru, which show variation in shells shape and are partly carinated, using both ecological and molecular data. The study area is situated in a valley at the western side of the Andes, with a total length of 25 km and an altitudinal range of almost 1,140 m, but focuses on two localities: El Infernillo at ca. 3,350 m and Tambo de Viso at ca. 2,700 m. The morphological variation of the species presents a rather complex picture that cannot completely be resolved by ecological data alone. Molecular data points to three taxa, two of which are variable in shell shape and each restricted to one of the localities mentioned; the third taxon is constant in shell shape and occurs over a large range of the study area. The species originally described as *Bostryx multiconspectus* Breure, 2008 and *B. primigenius* Breure, 2008 are redefined as a result.

Key words: carination, ecology, taxonomy, Peru, Orthalicoidea

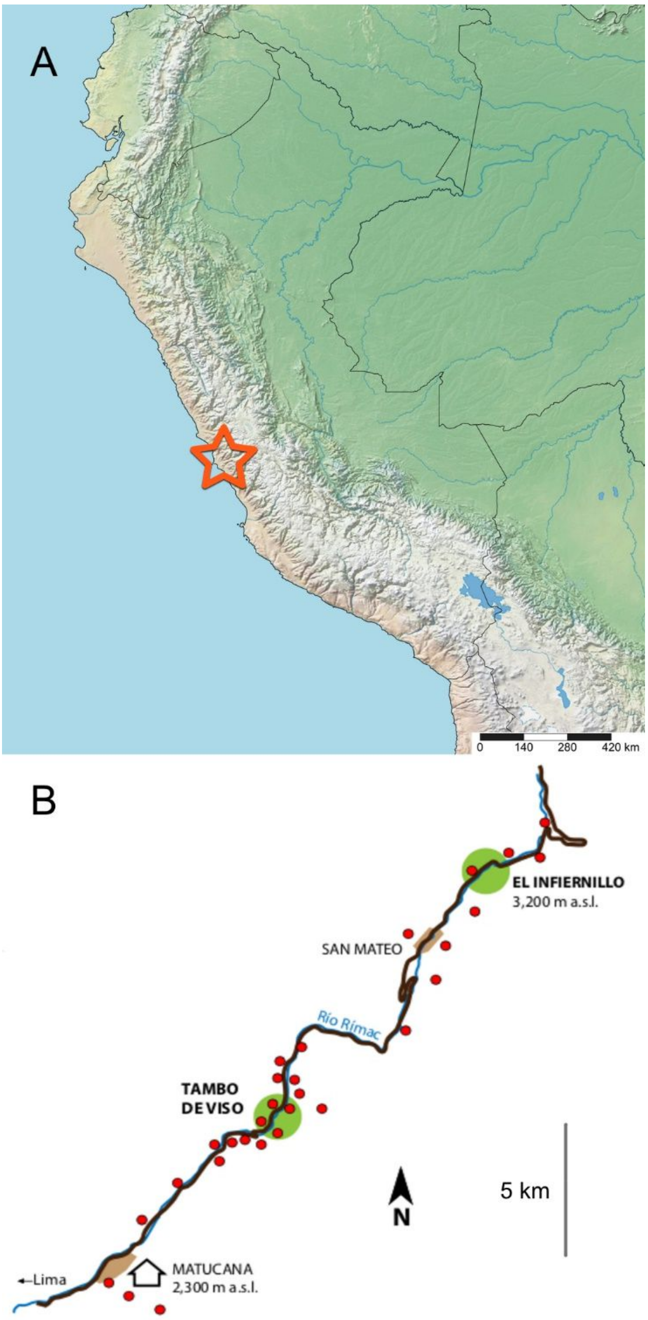
Résumé: Cet article traite des espèces de *Bostryx* du Pérou qui présentent des variations dans la forme de leur coquille et sont partiellement carénées, en utilisant à la fois des données écologiques et moléculaires. La zone d'étude est située dans une vallée du côté ouest des Andes, avec une longueur totale de 25 km et une étendue altitudinale de près de 1 140 m, mais se concentre sur deux localités : El Infernillo à env. 3 350 m et Tambo de Viso à env. 2 700 m. Les variations morphologiques des espèces présentent une évolution assez complexe que les seules données écologiques ne suffisent pas à expliquer. Les données moléculaires indiquent trois taxons, dont deux ont une forme de coquille variable et chacune est limitée à l'une des localités mentionnées ; le troisième taxon est constant dans la forme de la coquille et est présent sur une large étendue de la zone d'étude. Les espèces initialement décrites comme *Bostryx multiconspectus* Breure, 2008 et *B. primigenius* Breure, 2008 sont en conséquence redéfinies.

Mots clés : carination, écologie, taxinomie, Pérou, Orthalicoidea

INTRODUCTION

The Neotropical superfamily Orthalicoidea is a highly diverse and predominant group of land snails with currently almost 70 genera and more than 1,700 available species-level names. The group originated approximately 110 Ma ago (BREURE & ROMERO, 2012), and relict taxa of this group exist in South Africa, Melanesia, and Australia (Bothriembryontidae). Phylogenetic work on this superfamily is still scarce (WADE *ET AL.*, 2006; HERBERT & MITCHELL, 2009; BREURE *ET AL.*, 2010; BREURE & ROMERO, 2012) and additional research is likely to yield surprising results that will influence the classification of the families involved.

This paper is focuses on the genus *Bostryx* Troschel, 1847 belonging to the family Bulimulidae. Most *Bostryx* species are distributed from the high Andes (up to 3,600 m) down to coastal lowlands (BREURE, 1979), principally distributed in Peru, Chile, Bolivia and Argentina, but almost always highly restricted in geographical range. Thus, the rate of endemism at a regional or local scale is very high. In the Peruvian Andes most valleys are inhabited by different sets of species that were possibly separated during the folding of the Andes. Within *Bostryx* and some other Bulimulid genera a few species show a prominent morphological feature: carination (BREURE, 2008). This term has different definitions in malacological literature e.g., “having an angle rather than a round contour” (BURCH, 1962). In this paper we use the description by Breure (2008) “a carina is a somewhat outward protruding ridge, which may or may not internally be thickened. When a carina occurs both at the periphery and around the umbilicus of a shell, it is defined as doubly carinate”. Carination is a character that “strikes the eye” (Pilsbry & Olsson, 1949), but is not common in land snails, although it is found in different families around the world; in terrestrial snails notably in the Helicidae (Fiorentino *et al.*, 2008), and Camaenidae (Teshima *et al.*, 2003; Stankowski, 2011; Hirano *et al.*, 2015). However, carination also occurs in freshwater and marine species, making it likely that it repeatedly evolved in distinct lineages, thus being a “striking case of convergence” (Breure, 2008). In the species studied herein this phenomenon is combined with variation in shell shape. The shell may be depressed or discoidal, which some authors call “flat and keeled”, to elongate, or may even show detached whorls (Breure, 2008).



A Fig. 1. Study area in the valley of Río Rimac, Dept. Lima, Peru and its location. Red dots indicate the localities where more than 10 living individuals of *Bostryx primigenius* were collected (points can also indicate localities close together). The green shaded areas are the two areas where respectively *B. multiconspectus* and *B. solutus* have been found.

Fig. 2. Localities of *Bostryx multiconspectus*, mentioned as PN050 and PN058 in the text. A Site PN058 photographed from site PN050. B Site PN050 photographed from site PN058.

BREURE (2008) described two *Bostryx* species (*B. multiconspectus* and *B. primigenius*) from material collected by Weyrauch in the Río Rimac valley in Peru, one being flat and carinated, the other elongate without carination. Both species may be considered as sibling species or as one single species with a highly variable morphology. As *B. multiconspectus* was known only from one single site that had not been described in detail by Weyrauch on his collection labels, and therefore the species seemed to be a local endemic, whereas *B. primigenius* is reported from a larger part of the Río Rimac valley. So, *B. multiconspectus* could be suspected to be an ecotype of *B. primigenius*. In the same paper (BREURE, 2008) a population of *Bostryx solutus* (Troschel, 1847) was mentioned, which appeared to be cork-screw curled; this is aberrant compared to the typical shape as described by TROSCHEL (1847). The purpose of the current paper is to present additional data to elucidate the taxonomic status of these different shell shapes.

MATERIAL AND METHODS

Study area and organisms

The valley of the Río Rímac river is a long canyon valley between the towns of Chosica and Casapalca (Dept. Lima) situated on the western Andean slopes in Peru (Fig. 1), situated more or less SW–NE. Collection sites were between Matucana (2,450 m a.s.l.) and Río Blanco (3,590 m a.s.l.), with a total length of 25 km and an altitudinal range of almost 1,140 m. The study area is highly andine with rocky slopes or steep cliffs on both sides of the valley. Humidity is highest in the bottom of the valley and most plants can be found here, as well as land used for agriculture. The slopes are a dry and sparsely covered scrubland with many rock boulders. Mesoclimate is differing strongly throughout the study area forming wet and vegetation-rich patches spread over the valley and some moist side valleys. In the afternoons, the wind increasingly blows stronger from the southwestern lower parts of the valley, cooling down the area. The Río Rímac river and the Carretera Central (a highly frequented motorway that runs through the valley) are suspected to be strong barriers for snail dispersal leading to an effective separation of left and right side populations (Fig. 2). A railway track that runs parallel to both the river and the road, but does not seem to be a dispersal barrier for land snails. These three linear landscape elements cross each other several times along the valley forming long stretches of isolated areas in between. The valley is geologically diverse, containing outcrops of sedimentary (limestone, siltstone, mudstone, lutite, clay and conglomerates), metamorphic (quartzite, slate and schist), intrusive (e.g. granite) and volcanic rocks of various ages (Paleozoic to recent Quaternary) (CEPES, n.d.). *Bostryx multiconspectus* Breure, 2008 (Fig. 3, A-C), *B. primigenius* Breure, 2008 (Fig. 3, D-E), and *B. solutus* Troschel, 1847 (Fig. 4) all occur in the area, having been described from respectively Tambo de Viso (*B. multiconspectus*), and El Infernillo (*B. primigenius*, *B. solutus*).

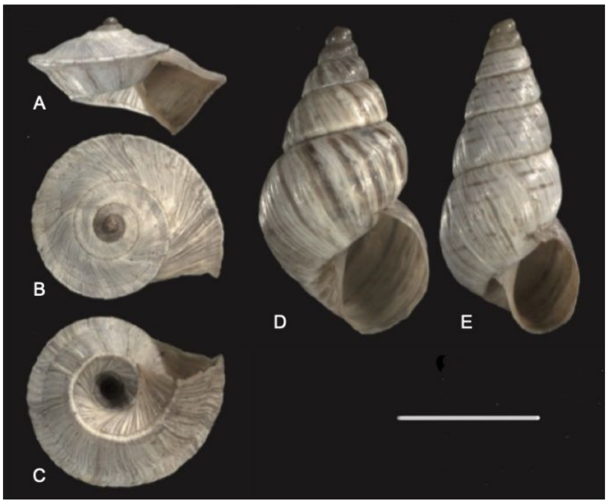


Fig. 3. *Bostryx* species used in this study. A-C, E *Bostryx multiconspectus* Breure, 2008, holotype SMF 156286 and specimen SMF 156285. D *Bostryx primigenius* Breure, 2008, holotype SMF 155706. Scale line 5 mm. Adapted from Breure, 2008.

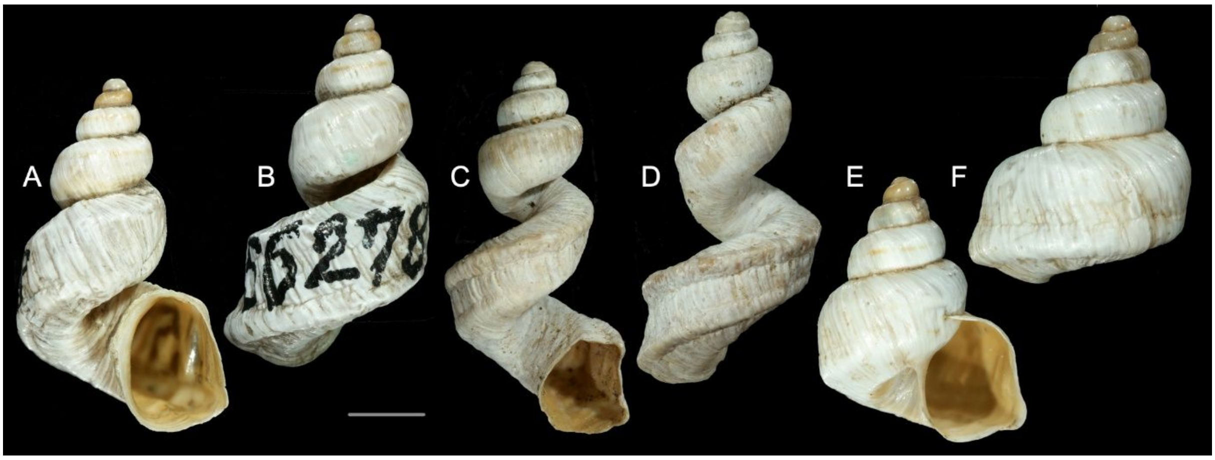


Fig. 4. *Bostryx* species used in this study. A-B *Bostryx solutus* (Troschel, 1847), typical of 'tight' form. C-D *Bostryx solutus* (Troschel, 1847), 'cork-screw' form. E-F *Bostryx solutus* (Troschel, 1847), 'compact' form. All material in SMF. Scale line 2 mm.

Field methods

We hand collected empty-shells of the three *Bostryx* species throughout the study area. Living specimens were sampled from several sites (10–12 individuals/site. These sites were scattered regarding altitude, river banks and distance from the river. Per site, different habitat parameters were assessed in the field (Supplementary data Fig. S1). A few shells of the carinated species *B. multiconspectus* were observed and collected at two different localities (PN050 and PN058, both at Tambo de Viso). In order to explore the potential influence of sunlight or wind exposition on carinated shell shapes, the exposition (in degrees) of the substrate surfaces was recorded for living individuals. GPS coordinates were taken with a GARMIN Dakota 20 device. Altitude was determined both by barometric measurements and GPS satellite signals. Dry material was stored in small plastic containers and foot muscle tissue was preserved in 96 % ethanol. Single specimens were drowned in water to relax their bodies and enable further genital preparations.

Microclimate analysis

To detect microclimatic differences between localities where *B. multiconspectus* occurs and those where only *B. primigenius* is found, we measured temperature and relative air humidity with data logger devices (Onset HOBO). This was done at two different sites, one of these at the left side of the river and the other one at the right side of the river. At each of these four measuring plots HOBO U23 Pro v2-002 devices were used to record air temperature and relative humidity at 10 minute intervals over a at least 20 day period. The data loggers were installed exactly in the position where living individuals of *B. multiconspectus* had been observed previously, and in nearby places where *B. primigenius* had been found.

We used the same data loggers to record the microclimatic conditions inside the transition zone of *B. solutus* at El Infiernillo, next to the motorway. The devices were placed at different points spread over the area. These points were selected in function of the possibility to camouflage and protect snails against human disturbance and strong wind. Logging parameters were the same as above. The logging data were analysed using the statistical software R and the programming environment software R Studio. Welch two sample t-tests, Shapiro-Wilk tests, and Wilcoxon signed rank tests were used to identify significant differences between the plot with *B. multiconspectus* and those where it is absent.

Phylogenetic analysis

Whole genomic DNA was extracted with DNeasy kit (Quiagen Inc.) following the manufacturer's protocol for animal tissue. PCR primers were LCO1490 and HCO2198 for CO1 (Folmer *et al*., 1994), 16Sar and 16Sbr for 16S-rRNA (Palumbi *et al.*, 1991), and LSU-1 and LSU-3 for the 3' end of 5.8S-rRNA, the ITS2 region, and the 5' end of 28S-rRNA gene (hereafter ITS2) (Wade & Mordan, 2000). Sequences were aligned using MAFFT as implemented in Geneious 7.1.3 (Biomatters Ltd.), and trimmed with Gblocks (Castresana, 2000). The substitution model selected by jModeltest 2.1 (Posada, 2008), using the Akaike Information Criterion, was HKY+G. Further analyses were done using MEGA7 (Kumar *et al.*, 2016) with its Maximum Likelihood (ML, bootstrap 500) procedure and with Bayesian inference (BI) using MrBayes 3.2.6 as implemented in Phylogeny.fr (Huelsenbeck & Ronquist, 2001; Dereeper *et al*., 2008). Two *Bothriembryon* species (Bothriembryontidae, Orthalicoidea; see Breure & Romero, 2012) were used as outgroup. Information on the material sequenced in this study is provided in Table 1. In order to improve the resolution of phylogenetic reconstruction, CO1 and 16S sequences were concatenated; the relatively small number of ITS2 sequences were analysed separately. In total there were 128 CO1 and 122 16S sequences. Identical sequences were discarded in the concatenated alignment. The resulting matrix consisted of 23 concatenated CO1/16S sequences for *Bostryx multiconspectus*/*B. primigenius* with a length of 1047 bp., and 26 concatenated CO1/16S sequences for *B. solutus* with a length of 1087 bp. The concatenated sequences were also used to construct haplotype networks with the software PopART 1.7 (Leigh & Bryant, 2015) using statistical parsimony networks as implemented in TCS (Templeton *et al.*, 1992).

Table 1. Material sequenced for this study. New sequences in GenBank are indicated in bold text. Locality codes are explaned in Table S1.

				GenBank accession numbers		
***S*pecies**	**Localitys**	**Collector**	**Voucher**	**CO1**	**16S**	**IT S2**
Bostryx multiconspectus Breure, 2008	Tambo de Viso (PN050)	P. Meinecke et al.	RMNH.MOL.201605			**MH465654**
Bostryx multiconspectus Breure, 2008	Tambo de Viso (PN050)	P. Meinecke et al.	RMNH.MOL.201606	**MH465639**	**MH465632**	
Bostryx multiconspectus Breure, 2008	Tambo de Viso (PN050)	P. Meinecke et al.	RMNH.MOL.201607	**MK455105**	**MK455145**	**MH465655**
Bostryx multiconspectus Breure, 2008	Tambo de Viso (PN058)	P. Meinecke, N. Richter-Harder	RMNH.MOL.PN058.4_1	**MK455106**	**MK455146**	
Bostryx primigenius Breure, 2008	San Mateo (PN043)	P. Meinecke, N. Richter-Harder	RMNH.MOL.201550	**MH465642**	**MK455147**	**MH465649**
Bostryx primigenius Breure, 2008	San Mateo (PN043)	P. Meinecke, N. Richter-Harder	RMNH.MOL.201552	**MK455107**	**MK455148**	
Bostryx primigenius Breure, 2008	Puente de Viso (PN052)	P. Meinecke et al.	RMNH.MOL.201553	**MH465643**	**MK455149**	**MH465650**
Bostryx primigenius Breure, 2008	Tambo de Viso (PN051)	P. Meinecke et al.	RMNH.MOL.201571	**MH465641**	**MH465627**	**MH465652**
Bostryx primigenius Breure, 2008	San Mateo (PN046)	P. Meinecke, N. Richter-Harder	RMNH.MOL.201572			**MH465653**
Bostryx primigenius Breure, 2008	Tambo de Viso (PN009)	P. Meinecke, N. Richter-Harder	RMNH.MOL.PN009_4	**MK455108**	**MK455150**	
Bostryx primigenius Breure, 2008	Tambo de Viso (PN011)	P. Meinecke, N. Richter-Harder	RMNH.MOL.PN011_3	**MK455109**	**MK455151**	
Bostryx primigenius Breure, 2008	Tambo de Viso (PN011)	P. Meinecke, N. Richter-Harder	RMNH.MOL.PN011_5	**MK455110**	**MK455152**	

Bostryx primigenius Breure, 2008	Tambo de Viso (PN012)	P. Meinecke, N. Richter-Harder	RMNH.MOL.PN012_1	**MK455111**	**MK455153**	
Bostryx primigenius Breure, 2008	Tambo de Viso (PN012)	P. Meinecke, N. Richter-Harder	RMNH.MOL.PN012_2	**MK455112**	**MK455154**	
Bostryx primigenius Breure, 2008	Tambo de Viso (PN012)	P. Meinecke, N. Richter-Harder	RMNH.MOL.PN012_4	**MK455113**	**MK455155**	
Bostryx primigenius Breure, 2008	Tambo de Viso (PN012)	P. Meinecke, N. Richter-Harder	RMNH.MOL.PN012_5	**MK455114**	**MK455156**	
Bostryx primigenius Breure, 2008	Tambo de Viso (PN014)	P. Meinecke, N. Richter-Harder	RMNH.MOL.PN014_1	**MK455115**	**MK455157**	
Bostryx primigenius Breure, 2008	Tambo de Viso (PN014)	P. Meinecke, N. Richter-Harder	RMNH.MOL.PN014_2	**MK455116**	**MK455158**	
Bostryx primigenius Breure, 2008	Tambo de Viso (PN014)	P. Meinecke, N. Richter-Harder	RMNH.MOL.PN014_4	**MH465644**	**MH465631**	
Bostryx primigenius Breure, 2008	Tambo de Viso (PN014)	P. Meinecke, N. Richter-Harder	RMNH.MOL.PN014_5	**MK455117**	**MK455159**	
Bostryx primigenius Breure, 2008	Tambo de Viso (PN017)	P. Meinecke, N. Richter-Harder	RMNH.MOL.PN017_3	**MK455118**	**MK455160**	
Bostryx primigenius Breure, 2008	San Mateo (PN018)	P. Meinecke, N. Richter-Harder	RMNH.MOL.PN018_1	**MK455119**	**MK455161**	
Bostryx primigenius Breure, 2008	Tambo de Viso (PN056)	P. Meinecke et al.	RMNH.MOL.PN056_1	**MK455120**	**MK455162**	
Bostryx primigenius Breure, 2008	Tambo de Viso (PN059)	P. Meinecke et al.	RMNH.MOL.PN059_1	**MH465645**	**MK455163**	
Bostryx primigenius Breure, 2008	Tambo de Viso (PN068)	P. Meinecke et al.	RMNH.MOL.PN068_2	**MK455121**	**MK455164**	
Bostryx solutus (Troschel, 1847)	El Infernillo (PN040)	P. Meinecke et al.	RMNH.MOL.201511	**MH465635**	**MH465628**	
Bostryx solutus (Troschel, 1847)	El Infernillo (PN040)	P. Meinecke et al.	RMNH.MOL.201512	**MK455122**	**MK455165**	
Bostryx solutus (Troschel, 1847)	El Infernillo (PN040)	P. Meinecke et al.	RMNH.MOL.201514	**MK455123**	**MK455166**	
Bostryx solutus (Troschel, 1847)	El Infernillo (PN048)	P. Meinecke et al.	RMNH.MOL.201523	**MK455124**	**MK455167**	
Bostryx solutus (Troschel, 1847)	El Infernillo (PN048)	P. Meinecke et al.	RMNH.MOL.201525	**MK455125**	**MK455168**	
Bostryx solutus (Troschel, 1847)	El Infernillo (PN039)	P. Meinecke et al.	RMNH.MOL.201530	**MK455126**	**MK455169**	**MH465648**
Bostryx solutus (Troschel, 1847)	El Infernillo (PN042)	P. Meinecke et al.	RMNH.MOL.201537	**MK455127**	**MK455170**	
Bostryx solutus (Troschel, 1847)	El Infernillo (PN042)	P. Meinecke et al.	RMNH.MOL.201538	**MK455128**	**MK455171**	
Bostryx solutus (Troschel, 1847)	El Infernillo (PN042)	P. Meinecke et al.	RMNH.MOL.201539	**MK455129**	**MK455172**	
Bostryx solutus (Troschel, 1847)	El Infernillo (PN042)	P. Meinecke et al.	RMNH.MOL.201540	**MK455130**	**MK455173**	
Bostryx solutus (Troschel, 1847)	El Infernillo (PN045)	P. Meinecke et al.	RMNH.MOL.201543	**MK455131**	**MK455174**	
Bostryx solutus (Troschel, 1847)	El Infernillo (PN045)	P. Meinecke et al.	RMNH.MOL.201547	**MK455132**	**MK455175**	
Bostryx solutus (Troschel, 1847)	El Infernillo (PN045)	P. Meinecke et al.	RMNH.MOL.201549	**MK455133**	**MK455176**	

Bostryx solutus (Troschel, 1847)	El Infernillo (PN041)	P. Meinecke et al.	RMNH.MOL.201556	**MK455134**	**MK455177**	
Bostryx solutus (Troschel, 1847)	El Infernillo (PN044)	P. Meinecke et al.	RMNH.MOL.201560	**MH465636**	**MK455178**	
Bostryx solutus (Troschel, 1847)	El Infernillo (PN044)	P. Meinecke et al.	RMNH.MOL.201561	**MK455135**	**MK455179**	
Bostryx solutus (Troschel, 1847)	El Infernillo (PN044)	P. Meinecke et al.	RMNH.MOL.201563	**MK455136**	**MK455180**	
Bostryx solutus (Troschel, 1847)	El Infernillo (PN044)	P. Meinecke et al.	RMNH.MOL.201565	**MK455137**	**MK455181**	
Bostryx solutus (Troschel, 1847)	El Infernillo (PN047)	P. Meinecke et al.	RMNH.MOL.201566	**MK455138**	**MK455182**	
Bostryx solutus (Troschel, 1847)	El Infernillo (PN047)	P. Meinecke et al.	RMNH.MOL.201568	**MK455139**	**MK455183**	**MH465651**
Bostryx solutus (Troschel, 1847)	El Infernillo (PN046)	P. Meinecke et al.	RMNH.MOL.201573	**MK455140**	**MK455184**	
Bostryx solutus (Troschel, 1847)	El Infernillo (PN046)	P. Meinecke et al.	RMNH.MOL.201575	**MK455141**	**MK455185**	
Bostryx solutus (Troschel, 1847)	El Infernillo (PN046)	P. Meinecke et al.	RMNH.MOL.201576	**MK455142**	**MK455186**	
Bostryx solutus (Troschel, 1847)	El Infernillo (PN046)	P. Meinecke et al.	RMNH.MOL.201577	**MK455143**	**MK455187**	
Bostryx solutus (Troschel, 1847)	El Infernillo (PN046)	P. Meinecke et al.	RMNH.MOL.201578	**MK455144**	**MK455188**	
Bostryx solutus (Troschel, 1847)	El Infernillo (PN039)	P. Meinecke et al.	RMNH.MOL.201604	**MH465637**	**MH465629**	
Bostryx solutus (Troschel, 1847)	El Infernillo	J.L. Ramírez	—			JQ669468
Bothriembryon glauerti	W. Australia, Stirling Range	M. Moir	WAM S34993	**MH465634**	**MH465626**	**MH465647**
Bothriembryon whitleyi	W. Australia, Geraldton	M. Moir & M. Lang	WAM S59466	**MH465633**	**MH465625**	**MH465646**

Abbreviations

The following abbreviations are used for the repositories of material: RMNH, Naturalis Biodiversity Center, Leiden, the Netherlands; SMF, Senckenberg Natur-Museum, Frankfurt am Main, Germany; ZMB, Museum für Naturkunde, Leibniz-Institut für Evolutions- und Biodiversitätsforschung, Humboldt Universität, Berlin, Germany.

RESULTS

Tissue sampling

In total 444 specimens and more than 1,660 empty shells were collected from 75 localities throughout the Río Rímac valley. These specimens belong mainly to the three study species but also to a few other species of the same genus and closely related genera (e.g., *Scutalus*).

We collected 356 specimens of *Bostryx primigenius* from 37 sites of which 31 sites yielded more than 10 individuals. At one site this species was found sitting on a vertical rock face at a distance of around 100 m from the nearest *B. multiconspectus* locality.

The carinated species *B. multiconspectus* was found at only two sites (PN050 and PN058), which face each other but are separated by the river and the motorway (Fig. 2). In total, 33 specimens and 189 empty shells were collected of this species. Almost all of the collected shells belonged to the flat form (Breure, 2008: fig. 3). Only at locality PN050 did we find conical shells with different degrees of carination. However, no living individuals of this form could be found. We suspect these snails to be living at higher elevations on the cliff that were not accessible to us. Site PN058, is situated adjacent to the village of Tambo de Viso. This is the original locality (Breure, 2012) sampled by Weyrauch, on which Breure (2008) based his type series.

Bostryx solutus is confined to the locality of El Infernillo, where the road bends sharply and is crossed at the same time by a railway bridge. Three different shell shapes are distinguished, ranging from a tightly coiled form (Fig. 4A-B), a detached form (Fig. 4C-D), to a compact form (Fig. 4E-F). The tight form was originally described by Troschel (1847) as this species, the two other forms were found in SMF with manuscript names by Weyrauch. In total we used 55 specimens for our DNA studies.

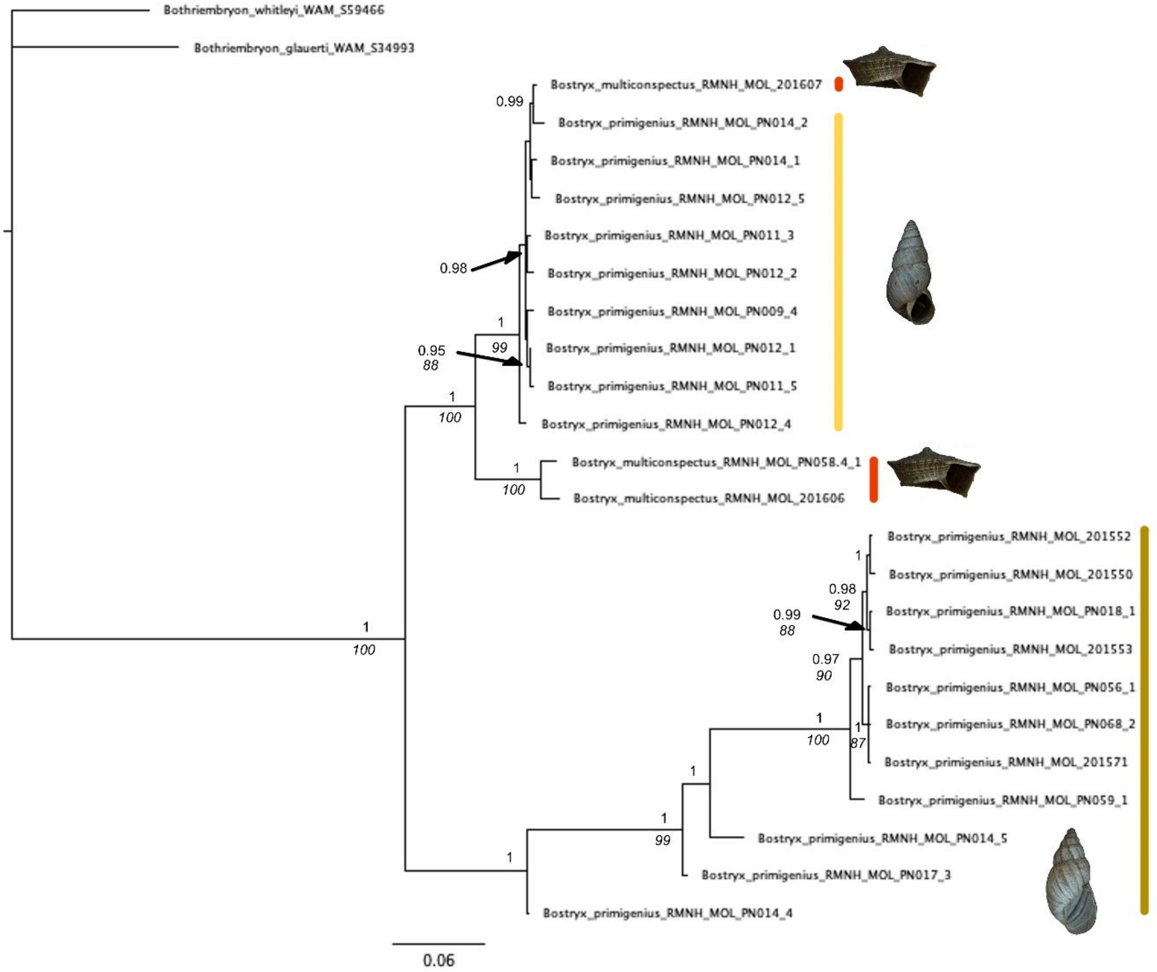


Fig. 5. Bayesian phylogeny of *Bostryx multiconspectus*/*B. primigenius* inferred by a MrBayes analysis, based a concatenated alignment of COI and 16S sequences (1047 bp). Posterior probabilities of ≥ 0.9 are shown at the left side of the nodes, italic numbers below refer to bootstrap support values (≥ 70) from a Maximum Likelihood analysis in MEGA7. Scale bar: substitutions/site. Colour codes correspond to those used in Fig. 6.

Ecological data

The four data loggers at Tambo de Viso generated a dataset during 19 days of comparable microclimatic data for temperature and relative humidity (Supplementary data, Fig. S1, Table S2). The two plots where *Bostryx multiconspectus* is absent show significantly higher mean temperatures than those where the species is present. While the overall minimum temperatures are almost equal, the overall maximum is also higher in the plots where this species is absent. Sites with *B. multiconspectus* had lower daily mean, minimum and maximum temperatures and lower daily temperature amplitudes, than comparable sites without *B. multiconspectus*.

Similar analyses were performed for the relative humidity data. The means, standard deviations and minimum values lie within the same range for all sets except plot 3 at locality PN058, where *B. multiconspectus* is present. Here, the relative humidity values are lower than in all other plots, whereas the standard deviation is higher, indicating a more scattered distribution of the recorded data. The maximum relative humidity at the four plots was 100 %. All value ranges were more or less constant among the different plots and sites except for plot 3. We cannot explain these aberrant data at plot 3 with our field observations.

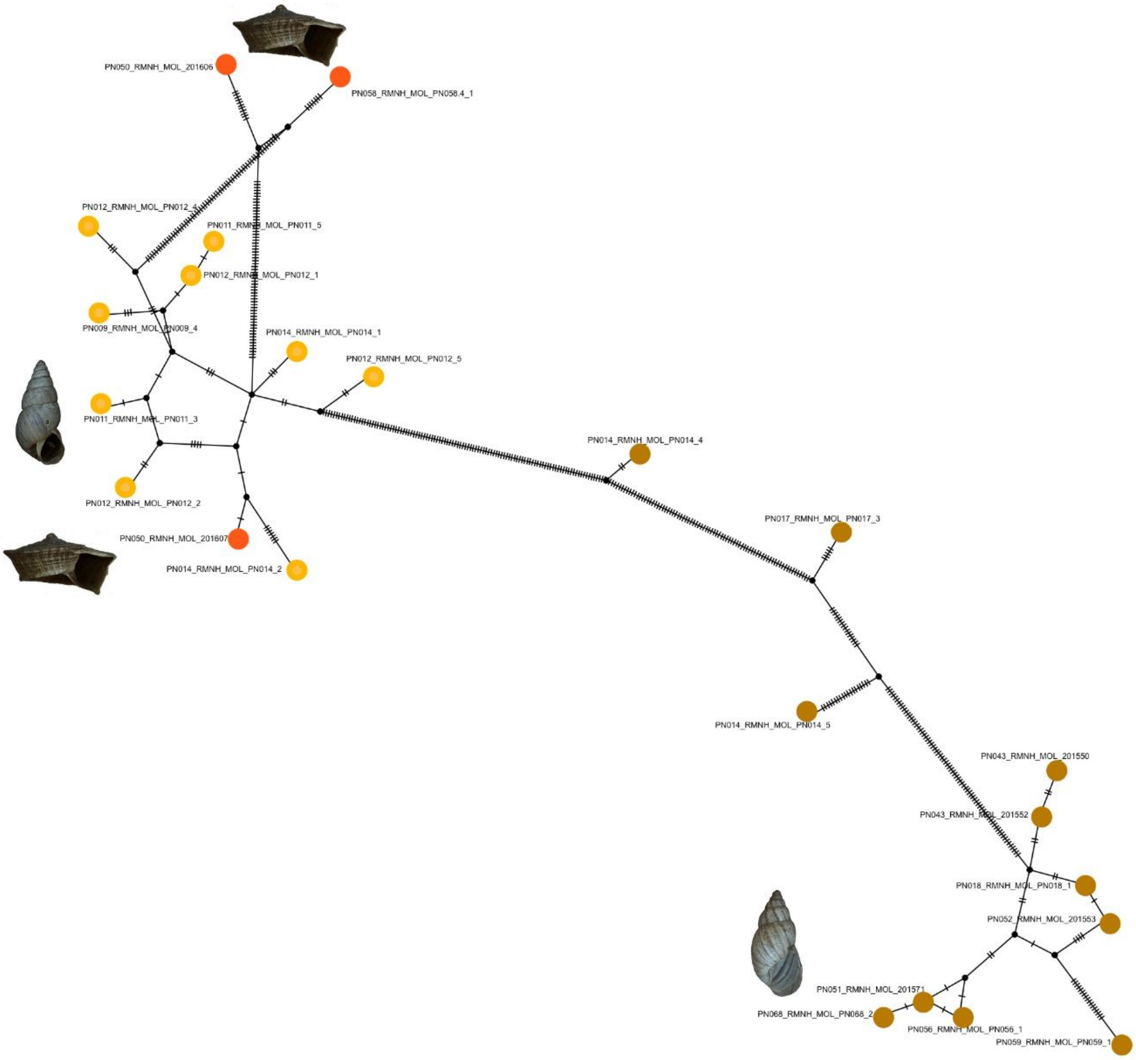


Fig. 6. Haplotype network for *Bostryx multiconspectus*/*B. primigenius* as result of a PopART analysis, based on the concatenated COI and 16S sequences (1047 bp). Hatch marks represent the number of mutations between haplotypes.

Phylogenetic mtDNA analyses: *Bostryx multiconspectus/B. primigenius*

Analyses of the concatenated alignment of CO1 and 16S (Fig. 5) showed two highly supported clades, both in ML (not shown) and BI. The largest clade comprises a mixture of morphs (depressed *multiconspectus* plus slender elongate *primigenius*) from near Tambo de Viso, while the other clade only contains the broadly elongate *primigenius* morph that occurs throughout the valley up to El Infernillo.

Phylogenetic mtDNA analyses: *Bostryx solutus*

Both the ML (not shown) and BI analyses of the concatenated CO1 and 16S alignment for this species show the same result: two well-supported clades; the largest one containing two subclades (Fig. 7). As a result all three morphs are separated.

Phylogenetic analyses: haplotype networks

Analysis of the concatenated CO1 and 16S alignment corroborates the division between the group of *Bostryx multiconspectus*/slender elongate *primigenius* and one hand and the relatively broadly elongate *B. primigenius* on the other hand. However, the number of mutations shows that some specimens, which according to the BI and ML analyses fall within the latter group, and in fact link the two groups (Fig. 6). A similar analysis for *B. solutus* shows the 'cork-screw' morph as a link between the typical and the compact morph (Fig. 8). Note that the spatial distance between the 'cork-screw' and the two other morphs is larger (ca. 200-700 meters, measured along the road) and its location is slightly lower (ca. 10-50 meter altitudinal difference) in the valley.

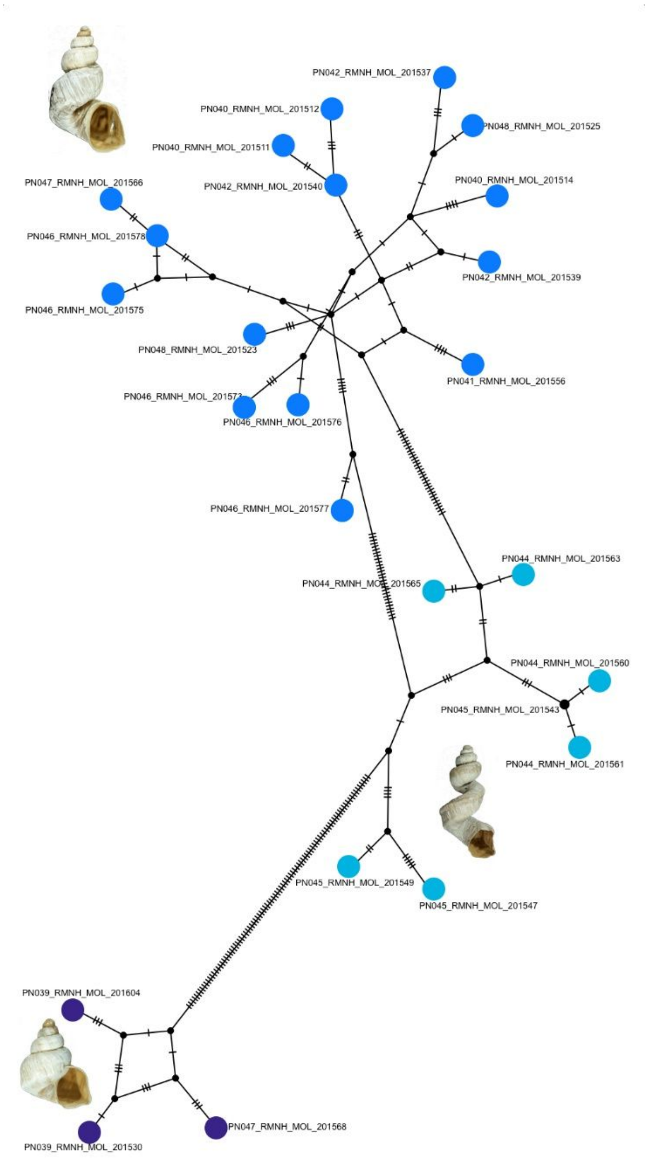


Fig. 7. Bayesian phylogeny for *Bostryx solutus* as result of a MrBayes analysis, based on a multilocus data set of mitochondrial DNA of 1087 bp. Posterior probabilities of ≥ 0.9 are shown at the left side of the nodes, italic numbers below refer to bootstrap support values (≥ 70) from a Maximum Likelihood analysis in MEGA7. Scale bar: substitutions/site. Colour codes correspond to those used in Fig. 8.

Phylogenetic analysis of ITS2

For the ITS2 analysis only a small number of specimens was available, which is the reason why all three *Bostryx* species were combined (Fig. 9). A BI analysis resulted in a paraphyletic structure for *Bostryx solutus*, including one sequence taken from GenBank; we were unable to check the morphotype of the voucher. *Bostryx multiconspectus* and *B. primigenius* were differentiated in two distinct clusters, one with representatives of shells with relatively short spire and large aperture, the other with a mixture of *B. solutus*, and *B. multiconspectus* plus the slender elongate *primigenius* morph. Note that ITS2 is less variable than mtDNA and more suited for analyses deeper in a phylogeny; which is the reason why we refrain from any conclusions at this stage.

DISCUSSION

Distribution of *Bostryx multiconspectus, B. primigenius* and *B. solutus*

As *Bostryx multiconspectus* and *B. solutus* only seem to occur at the sites reported here which is further verified by the literature, both species are probably narrow endemics in this area. To our knowledge *B. primigenius* is endemic to the valley of Río Rímac. The proximity of the three populations to the highly frequented motorway implies that these species might not be strongly affected by this degree of human impact.

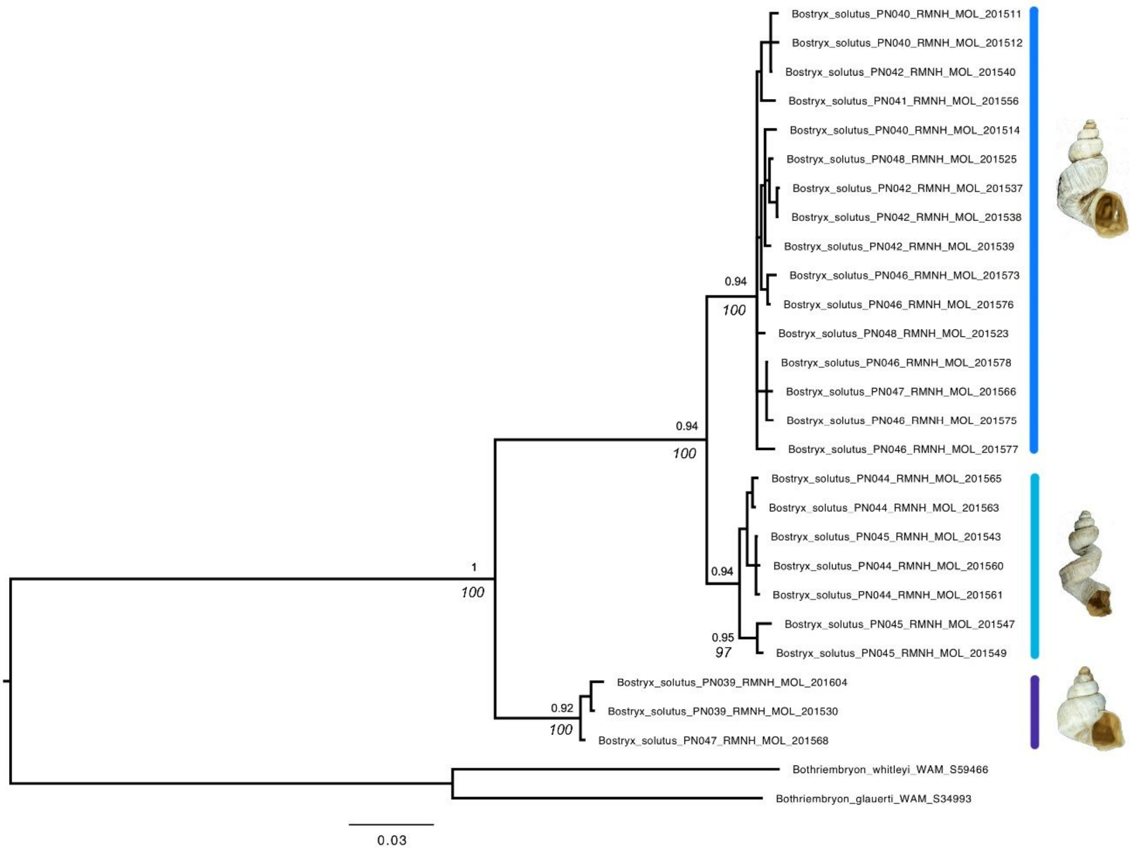


Fig. 8. Haplotype network for *Bostryx solutus* as result of a PopART analysis, based on the concatenated COI and 16S sequences (1087 bp). Hatch marks represent the number of mutations between haplotypes.

Correlating habitat characters

The analysis of microclimatic data showed that mean temperatures, daily maximum temperatures, daily minimum temperatures, and variation in daily temperatures at sites where *Bostryx multiconspectus* is present, are significantly lower than at sites where the species was absent. In contrast, there were no consistent differences for relative humidity. These findings do neither disagree, nor confirm the assumptions of Goodfriend (1986), who considered carination as an adaptation to dryer habitats. The cooler microclimate of these substrates can be a consequence of the chilling winds in the afternoons. We found *B. multiconspectus* resting inside of rock crevices probably hiding from wind, sunlight or predators. As we found *B. multiconspectus* only at steep to vertical rock faces or in short distance from or at the bottom of those (Fig. 10), we may assume an association between carinated forms and rocky habitats as other authors have shown before (e.g., Stankowski, 2011). *B. primigenius* instead was found mainly dwelling on shrubs (*Ambrosia* spp. and others) throughout the study area (Fig. 11), in several cases relatively close to rocks and cliffs.

Transition series

Unfortunately we were not able to find living specimens of the intermediate forms of the *B. multiconspectus* transition series. This makes it more difficult to explore potential genetic gradients both inside *B. multiconspectus* and between this species and *B. primigenius*. As we could find empty and broken shells of intermediate forms (cf. Breure 2008: fig. 4) at site PN050 we assume that there might be living individuals around that site. We suppose that they live at higher elevations of the rock faces where sampling was not possible. A more detailed study using microsatellite DNA would be necessary to investigate this putative hybrid zone of the two morphotypes within *B. multiconspectus* (cf. Stankowski, 2013). Johnson & Stankowski (2018) recently showed similar clines in *Rhagada* species from Rosemary Island, previously thought to form different species, were actually a sign of morphological continuity.

GOMPERT *et al*. (2017) have recently reviewed the literature on hybrid zones and how different types of genomic data contribute to our understanding of these zones. When we view our results from a spatial perspective, the idiosyncratic distribution of hybrid populations in the Río Rimac valley may perhaps be best characterised as mosaic hybrid zones.

Phylogeny and taxonomy

The topologies of the trees and networks all point to the distinction of three different species groups which is strongly supported in most phylogenetic analyses using the variable mitochondrial genes, but less so in a much more restricted sample of the less variable nuclear gene. The complex of *Bostryx primigenius* and *B. multiconspectus* is differentiated in two groups. One group, with relatively short spire and large aperture (Fig. 3D: *Bostryx primigenius*), occurring from higher above El Infernillo down into the valley below Tambo de Viso, is always elongate in shell shape. The other group has shells with a relatively slender spire and small aperture (Fig. 3E), but comprises also different shell shapes: *Bostryx multiconspectus*, which is confined to the Tambo de Viso area, can have both elongate or depressed and carinate shells depending on the microhabitat, while transitional shapes also do occur. Finally, the third species group which is *Bostryx solutus*, confined to El Infernillo, may be found in three morphs: the tightly coiled shape as originally described, a detached 'cork-screw' shape (with transitional shapes between these two), and a compact shape.

The classification of the three taxa discussed is thus (partly emended from BREURE, 2008):

***Bostryx multiconspectus* Breure, 2008**

Bostryx multiconspectus Breure, 2008: 502, figs 3-4, 6.
Type locality: Peru, Dept. Lima, Río Rimac valley, Tambo de Viso, 2700 m.
Type material: All material listed in BREURE (2008: 502).
Additional (non-type) material: Tambo de Viso (SMF 156285/1, 156279/1, 155597/1, 155598/1, 155599/1, 162089/1).
Remarks: Contrary to Breure (2008), the slender elongate morph (and the transitional ones with the depressed and carinated morph) is now considered as part of this species. This species is highly restricted to two spots opposite each other, divided by the road, railroad and the river. It occurs both in rock crevices, on rock ledges, and on shrubs nearby (Fig. 10). The additional material above was incorrectly assigned by Breure (2008) to the next species.

***Bostryx primigenius* Breure, 2008**

Bostryx primigenius Breure, 2008: 505, fig. 5.
Type locality: Peru, Dept. Lima, right bank Rio Rímac, El Infernillo, 3360 m.
Type material: All material listed in BREURE (2008: 502), excluding Tambo de Viso (SMF 156285/1, 156279/1, 155597/1, 155598/1, 155599/1, 162089/1).
Remarks: This species is now restricted to the elongate morph, which is relatively broad (most noticeable in the last whorls) and has a larger aperture, occurring throughout the valley from around El Infernillo down to below Tambo de Viso. It can typically be found only on shrubs (Fig. 11).

***Bostryx solutus* (Troschel, 1847)**

Bulimus (*Bostryx*) *solutus* Troschel, 1847: 49.
Type locality: Peru.
Type material: Lectotype ZMB 10252.
Remarks: This species is treated as one, despite the fact that the three morphs in the analysis of the mitochondrial DNA are neatly split in (sub)clades. The number of specimens analysed with ITS2, however, is too small to draw any conclusion at this stage. Further research on additional specimens may be needed. It occurs on steep rocks (Fig. 12).

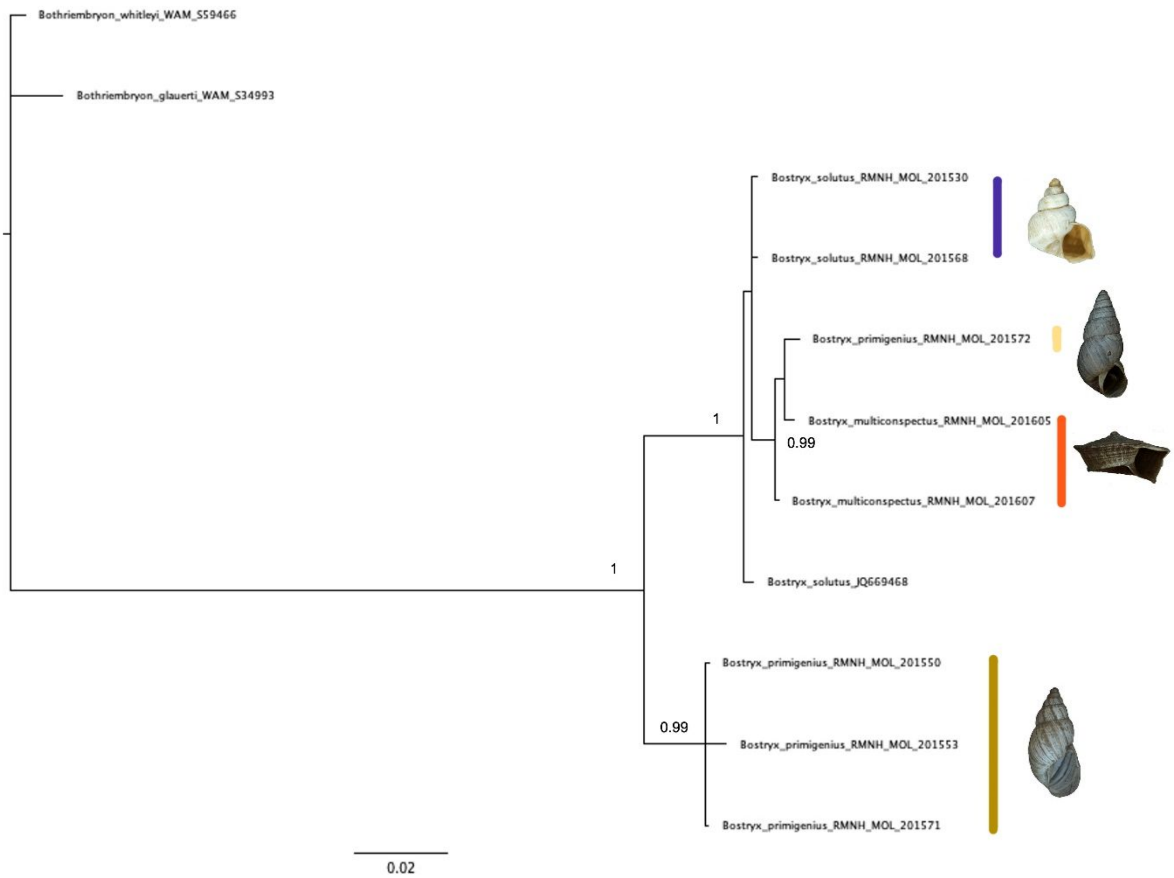


Fig. 9. Bayesian phylogeny for *Bostryx multiconspectus*/*B. primigenius* and *B. solutus* as result of a MrBayes analysis, based on a data set of nuclear DNA (ITS2). Scale bar: substitutions/site. Colour coding as in Figs 5-8.

Fig. 10. *Bostryx multiconspectus* in its natural habitat. A Individual resting inside a crevice at site PN058. B Individual extended from shell on mosses at site PN050. For size, compare with Fig. 3A-C.

Fig. 11. *Bostryx primigenius* in its natural habitat.

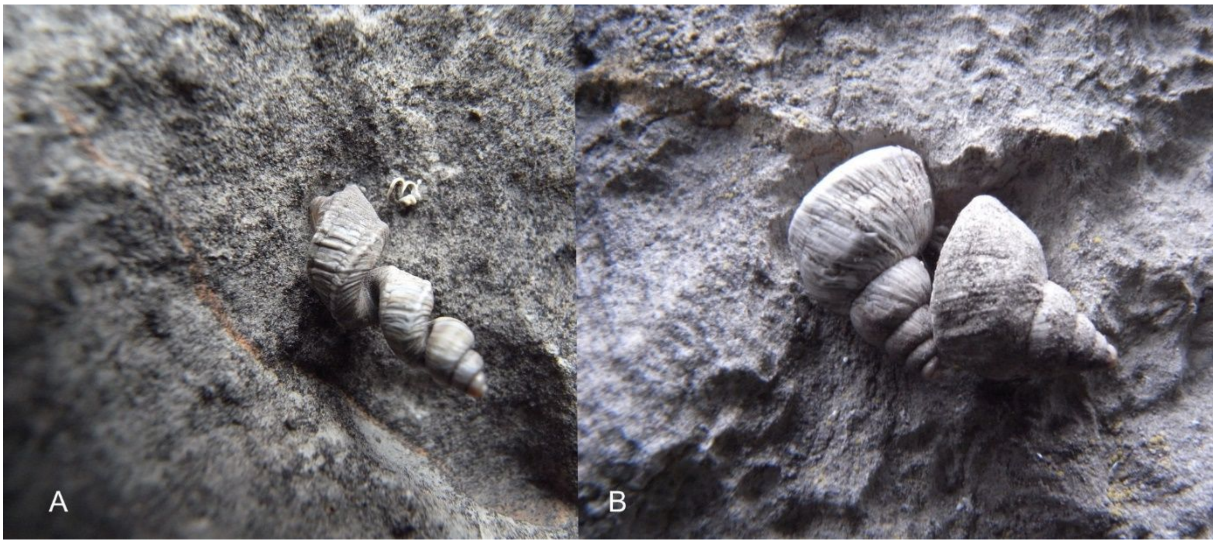


Fig. 12. Different shell shapes in *Bostryx solutus* in natural habitat. A Detached shell shape ('cork screw'). B Compact shell shape.

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SUPPLEMENTARY DATA

The following data files are available at : **https://doi.org/10.6084/m9.figshare.7564877.v1Fig. S1**
Data on temperature and humidity derived from data loggers (after MEINECKE, 2013). [PDF-file]
Table S1. Data for localities, samples, dataloggers, and habitats. [XLSX-file]
Table S2. Analyses on data logger data related to temperature and humidity (after MEINECKE, 2013). [PDF-file]