Orobothriurus (Scorpiones: Bothriuridae) phylogeny, Andean biogeography, and the relative importance of genitalic and somatic characters

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The genus *Orobothriurus* Maury, 1976 (Bothriuridae Simon, 1880) displays an Andean pattern of distribution, most of its species occurring at high altitudes (over 2000–2500 m to a maximum altitude record of 4910 m) from central Peru to Argentina. The recent discovery of several new species and the uncertain phylogenetic position of *Orobothriurus lourencoi* Ojanguren Affilastro, 2003, required a reanalysis of *Orobothriurus* phylogeny. Thirty bothriurid taxa, including all species of *Orobothriurus* and *Pachakutej* Ochoa, 2004, were scored for 65 morphological characters and analysed with parsimony under equal and implied weighting. The resulting topology justifies the establishment of a new genus, *Rumikiru* Ojanguren Affilastro *et al.*, in press, for *O. lourencoi* and a closely related, new species, *Rumikiru atacama* Ojanguren Affilastro *et al.*, in press. It also offers new insights about the phylogeny and biogeography of *Orobothriurus* and related genera. Characters from the male genitalia (i.e. hemispermatophore), comprising approximately 26% of the morphological matrix, were found to be less homoplastic than those from somatic morphology, contradicting suggestions that genitalia are uninformative or potentially misleading in phylogenetic studies.

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Introduction

The scorpion genus *Orobothriurus* Maury, 1976 was established to accommodate several small to medium-sized bothriurid scorpions, endemic to the Andes and surroundings in Peru, Chile and Argentina (Fig. 1), and previously placed in *Bothriurus* Peters, 1861. At the time of its creation, Maury (1976) recognized eight species in *Orobothriurus*: *O. alticola* (Pocock, 1899), type species of the genus; *O. curvidigitus* (Kraepelin, 1911); *O. peruvianus* (Mello-Leitão, 1948); *O. dumayi* (Cekalovic, 1974); *O. crassimanus* Maury, 1976; *O. inca* Maury, 1976; *O. parvus* Maury, 1976.

Maury (1976) recognized two species groups in *Orobothriurus*, subsequently named the *alticola* group and the *inca* group (Acosta & Ochoa 2001), based on marked differences in somatic and genital morphology.

No further mention was made about the systematics of *Orobothriurus* for 25 years until its monophyly was questioned in larger analyses of scorpionoid and bothriurid phylogeny (Prendini 2000, 2003a). Subsequent works on the genus led to various taxonomic changes and additions. *Orobothriurus dumayi* was returned to *Bothriurus* (Acosta & Ochoa 2001) and six new species were described (Acosta
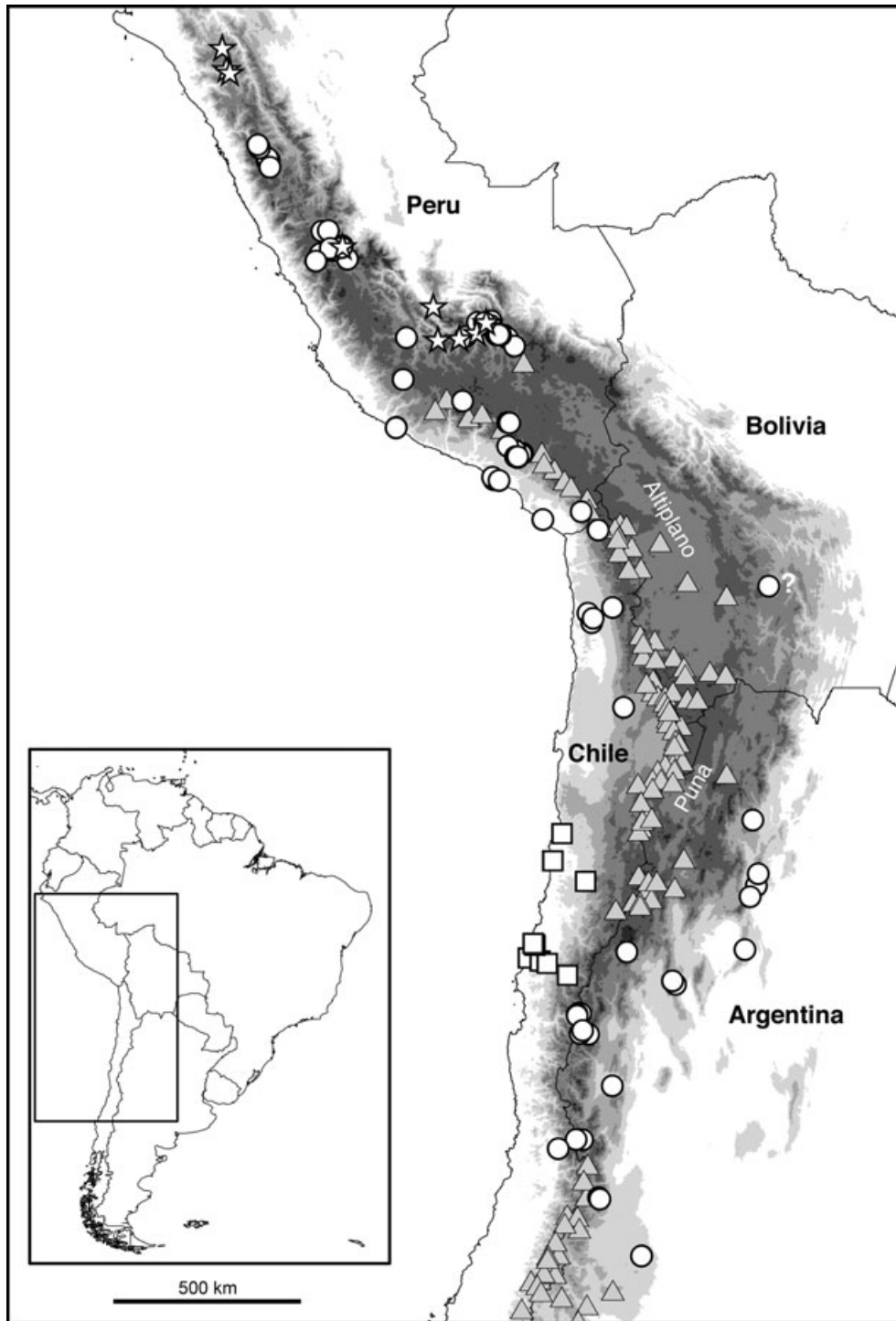
Fig. 1 Map of western South America, plotting known records of all species of the bothriurid scorpion genera *Orobothriurus* Maury, 1976 (circles), *Pachakutej* Ochoa, 2004 (stars) and *Rumikiru* Ojanguren Affilastro *et al.*, in press (squares), topography (contour interval 1000 m) and volcanoes (triangles). Single record for *Orobothriurus wawita* Acosta & Ochoa, 2000 from Bolivia (indicated by questionmark) probably due to mislabeling (Ochoa *et al.* 2011).

& Ochoa 2000, 2001; Ochoa & Acosta 2002, 2003; Ojanguren Affilastro 2003a): *O. wawita* Acosta & Ochoa, 2000; *O. famatina* Acosta, 2001; *O. iskay* Acosta & Ochoa, 2001; *O. atiquipa* Ochoa & Acosta, 2002; *O. ampay* Ochoa & Acosta, 2003; and *O. lourencoi* Ojanguren Affilastro, 2003.

One of these species, *O. lourencoi*, from the coastal Atacama Desert in Chile, possesses several characters unique among bothriurid scorpions, including an enlarged basal tooth on the median denticle row of the pedipalp chela movable finger and an apophysis situated submedially on the internal surface of the pedipalp chela of the adult male (Ojanguren Affilastro 2003a).

Ochoa (2004) revised *Orobothriurus* and presented a cladistic analysis based on 57 morphological characters scored for the 11 species known at the time. Ochoa's (2004) results supported Prendini's (2000, 2003a) prediction that *Orobothriurus* was paraphyletic, the *inca* group being monophyletic with *Bothriurus* to the exclusion of the *alticola* group. Ochoa (2004) established the genus *Pachakutej* Ochoa, 2004 to accommodate the species of the *inca* group and transferred four species from *Orobothriurus*: *P. peruvianus* (Mello-Leitão, 1948); *P. crassimanus* (Maury, 1976); *P. inca* (Maury, 1976); *P. iskay* (Acosta & Ochoa, 2001). In addition, two new species were described: *P. juchuicha* Ochoa, 2004; *P. oscari* Ochoa, 2004. Following several expeditions to the Andes of Peru, Chile and Argentina, seven additional species of *Orobothriurus* were described (Ojanguren Affilastro *et al.* 2009; Ochoa *et al.* 2011) and one was synonymized (Ojanguren Affilastro *et al.* 2009), bringing the total species count to 16 (Table 1).

As redefined by Ochoa (2004), the species of *Orobothriurus* share several synapomorphies: a narrow, elongated hemispermatophore, with a poorly developed lobe region, a divided frontal crest, an enlarged basal lobe with a tortuous stem and a subdistal spoon-like dilation ending in a terminal process; an acute apophysis on the internal surface of the pedipalp chela manus of the adult male, with the *ib* trichobothrium situated at its base; the close proximity and subparallel orientation of the ventrosubmedian carinae to the ventrolateral carinae on metasomal segment V.

Ochoa (2004) omitted *O. lourencoi* from his analysis, so this enigmatic species remained in *Orobothriurus* by default, pending an empirical evaluation of its phylogenetic placement. *Orobothriurus lourencoi* exhibits several characters that suggest a closer relationship to *Pachakutej* (e.g. hemispermatophore with a papillose fold on the basal lobe and a sclerotized apophysis on the internal fold of the internal lobe), but resembles the species of *Orobothriurus* in other respects (e.g. the carination of metasomal segment V; Ojanguren Affilastro 2003a: p. 119, fig. 9).

The discovery of a new species, closely related to *O. lourencoi*, in the Atacama Desert of Chile (Ojanguren

Table 1 Described species of the bothriurid scorpion genera *Orobothriurus* Maury, 1976, *Pachakutej* Ochoa, 2004 and *Rumikiru* Ojanguren Affilastro *et al.*, in press, with countries and provinces (Argentina), regions (Chile) or departments (Peru) from which they have been recorded

Orobothriurus alticola (Pocock, 1899)	Argentina: Mendoza, San Juan
Orobothriurus ampay Ochoa & Acosta, 2003	Peru: Apurímac
Orobothriurus atiquipa Ochoa & Acosta, 2002	Peru: Arequipa
Orobothriurus calchaqui Ochoa et al., 2011	Argentina: Tucumán
Orobothriurus compagnuccii Ochoa et al., 2011	Argentina: La Rioja
Orobothriurus curvidigitus (Kraepelin, 1911)	Peru: Arequipa
Orobothriurus famatina Acosta, 2001	Argentina: La Rioja
Orobothriurus grismadoi Ojanguren Affilastro et al., 2009	Argentina: Mendoza
Orobothriurus huascaran Ochoa et al., 2011	Peru: Ancash
Orobothriurus paessleri (Kraepelin, 1911)	Peru: Arequipa
Orobothriurus parvus Maury, 1976	Peru: Junín, Lima
Orobothriurus quewerukana Ochoa et al., 2011	Chile: Tarapacá, Peru: Tacna
Orobothriurus ramirezi Ochoa et al., 2011	Chile: Coquimbo
Orobothriurus tamarugal Ochoa et al., 2011	Chile: Tarapacá
Orobothriurus wawita Acosta & Ochoa, 2000	Peru: Ayacucho, Cusco
Pachakutej crassimanus (Maury, 1976)	Peru: Cajamarca
Pachakutej inca (Maury, 1976)	Peru: Cajamarca
Pachakutej iskay (Acosta & Ochoa, 2001)	Peru: Apurímac, Cusco
Pachakutej juchuicha Ochoa, 2004	Peru: Cusco
Pachakutej peruvianus (Mello-Leitão, 1948)	Peru: Junín
Pachakutej oscari Ochoa, 2004	Peru: Cusco
Rumikiru atacama Ojanguren Affilastro et al., in press	Chile: Atacama
Rumikiru lourencoi (Ojanguren Affilastro, 2003)	Chile: Antofagasta, Atacama

Affilastro *et al.* in press), raised further questions about the phylogenetic placement of the two species. A reanalysis of *Orobothriurus* phylogeny was therefore performed, using a revised, expanded version of Ochoa's (2004) data matrix, based on 17 species of *Orobothriurus* (Ojanguren Affilastro *et al.* 2009; Ochoa *et al.* 2011), including *O. lourencoi*, omitted from Ochoa's (2004) analysis; the closely related, new species; all species of *Pachakutej*; and a selection of other bothriurid taxa. Results of this analysis justify the establishment of a new genus, *Rumikiru* Ojanguren Affilastro *et al.*, in press, for *O. lourencoi* and the new species, *Rumikiru atacama* Ojanguren Affilastro *et al.*, in press, and offer new insights about the phylogeny and biogeography of *Orobothriurus* and related genera.

Approximately 26% of the morphological characters used in the analysis are derived from male genitalia (i.e. hemispermatophore). Bothriurid scorpions exhibit some of the greatest diversity in male genitalia among scorpions (Maury 1980; Ojanguren Affilastro 2005; Peretti 2010). It has been argued that genital characters of arthropods evolve relatively rapidly, because of strong sexual selection, and have low phylogenetic inertia (Arnqvist & Rowe 2002;

Eberhard 2004). This implies that genital characters could be highly homoplastic and therefore misleading, or at least uninformative, in phylogenetic studies. Recently, Song & Bucheli (2010) provided the first empirical assessment of this assumption, showing that genitalic and somatic characters have similar levels of homoplasy in insects. Similar comparisons have not been conducted in arachnids, which have sclerotized genitalia like insects. We therefore present a comparison of homoplasy among genitalic and somatic characters in bothriurid scorpions.

Material and methods

Taxa

All 16 described species of *Orobothriurus* and one undescribed species were included as ingroup taxa in the present analysis (Table 2). The outgroup taxon sample comprised 13 species in five bothriurid genera. All six described species of *Pachakutej* were included to test their relationship with *Rumikiru*. Four species of *Bothriurus* were included, based on previous findings of a close relationship with species of *Pachakutej* (Prendini 2000, 2003a; Ochoa 2004): *Bothriurus asper* Pocock, 1893; *Bothriurus bonariensis* (C.L. Koch, 1842); *Bothriurus cordubensis* Acosta, 1995; *Bothriurus coriaceus* Pocock, 1893. Three exemplar species of other, more distantly related bothriurid genera were also included: *Centromachetes pocockii* (Kraepelin, 1894); *Cercophonius sulcatus* Kraepelin, 1908; *Urophonius tregualemuensis* Cekalovic, 1981. The tree was rooted on *C. sulcatus*, based on previous evidence for the relationships among bothriurid genera (Prendini 2000, 2003a).

Material examined

Specimens were collected by turning stones during the day or by ultraviolet (UV) light detection at night (Honetschlager 1965; Stahnke 1972; Sissom *et al.* 1990). Portable UV lamps, comprising two mercury-vapour tubes attached to a chromium parabolic reflector and powered by a rechargeable 7 Amp/h, 12 V battery, or Maglite© 3D cell

Table 2 Distribution of 65 characters among 30 bothriurid scorpion species, including all species of *Orobothriurus* Maury, 1976, *Pachakutej* Ochoa, 2004 and *Rumikiru* Ojanguren Affilastro *et al.*, in press

	0	5	10	15	20	25	30	35	40	45	50	55	60
Cercophonius sulcatus	00110	00010	10000	00201	10000	01110	01100	00000	00111	0110–	1010–	—010	0—00
Urophonius tregualemuensis	21011	00010	10000	00201	10001	11111	01100	11100	00011	0110–	1010–	—010	0—00
Centromachetes pocockii	00110	00011	01001	00300	10020	00001	01100	00000	00001	0000–	1000–	—010	0—00
Bothriurus asper	10110	00101	01110	00000	00120	01100	00011	11110	20000	00100	00010	0–000	0—00
Bothriurus bonariensis	00110	00101	01110	00000	00121	01100	00011	11110	20000	00010	02010	2–000	0—00
Bothriurus cordubensis	10110	00111	01110	00000	00101	01100	01111	11110	20000	0000–	0110–	—110	0—00
Bothriurus coriaceus	00000	00111	01110	00000	00101	01100	01111	11100	20000	0000–	0110–	—110	0—00
Pachakutej crassimanus	00110	00111	01000	00000	00000	00000	10011	11110	10001	01110	02010	0–101	0—11
Pachakutej inca	00110	00110	01000	00000	00000	00000	10011	11120	01001	01110	02010	0–101	0—11
Pachakutej iskay	00110	00111	01000	00000	00000	00000	10011	11110	10001	01110	02010	0–101	0—11
Pachakutej juchuicha	00111	00111	01000	00000	00000	00000	10011	11120	01001	01110	02010	0–101	0—11
Pachakutej oscari	00110	00111	01000	00000	00000	00000	10011	11110	10001	01110	01010	0–101	0—11
Pachakutej peruvianus	00110	00111	01000	00000	00000	00000	10111	11100	10001	01110	02010	0–101	0—11
Rumikiru atacama	0–110	10111	01000	10410	01201	10100	10011	11101	00111	00010	22010	0–101	0—01
Rumikiru lourencoi	0–110	10111	01000	10410	01201	10100	10011	11101	00111	00010	22010	0–101	0—01
Orobothriurus alticola	11101	00010	10000	00100	00112	11110	11100	11101	00111	$1101	12011	11000	11100
Orobothriurus ampay	00110	00010	10000	01–00	00111	11100	11100	00001	00111	11100	12011	10000	11000
Orobothriurus atiquipa	11101	00010	1–000	00100	00112	11110	10??1	??10–	00111	11201	12011	11000	11100
Orobothriurus calchaqui	11101	00010	10000	00100	00112	11110	11100	01101	00111	01100	12011	10000	12000
Orobothriurus compagnuccii	11101	00010	10000	00100	0011&	11110	11101	11101	00111	01100	12011	11000	11100
Orobothriurus curvidigitus	11101	01010	10001	10100	00122	11110	10011	11101	00111	01100	12011	11000	11100
Orobothriurus famatina	11001	00010	10000	00100	00111	11110	11100	01101	00111	01100	12011	10000	12000
Orobothriurus grismadoi	10101	00010	10000	00100	00112	11110	11101	11101	0–111	11101	12011	11000	11100
Orobothriurus huascaran	11111	00010	10000	00100	00122	11010	11100	01101	00111	01201	12011	11000	11100
Orobothriurus paessleri	11101	01010	11000	10100	00122	11110	10011	11101	00101	11100	12011	11000	11100
Orobothriurus parvus	11011	00010	10000	00100	00111	11110	11100	01101	00111	01200	12011	10000	10000
Orobothriurus quewerukana	11101	01010	11001	10100	00112	11110	10011	11101	00111	01100	12011	11000	11100
Orobothriurus ramirezi	11101	00010	10000	00100	00112	11110	11100	11101	0–111	01101	12011	11000	11100
Orobothriurus tamarugal	11002	01010	11001	10100	00111	11110	10111	11101	0–101	01100	12011	11000	11100
Orobothriurus wawita	11012	00010	10000	01–00	00–11	11110	10101	1112–	01111	01200	12011	10000	11000

Character states are scored 0–4, ? (unknown) or - (inapplicable); polymorphic states: $ = [0 1]; & = [1 2]. Refer to Appendix 1 for character descriptions and Appendix S1 for material examined.

torches with 32 UV LED attachments were used for field-work. Materials examined (Appendix S1) are deposited in the following collections: American Museum of Natural History, New York, USA; Cátedra de Diversidad Animal I, Universidad Nacional de Córdoba, Argentina; Field Museum of Natural History, Chicago, USA; Instituto Miguel Lillo, Tucumán, Argentina; Laboratorio de Biología Reproductiva y Evolución, Universidad Nacional de Córdoba, Argentina; Museo Argentino de Ciencias Naturales 'Bernardino Rivadavia,' Buenos Aires, Argentina; Museo Nacional de Historia Natural, Santiago, Chile; Museo de Historia Natural, Universidad Nacional de San Antonio Abad del Cusco, Peru; Museo de Historia Natural, Universidad Nacional Mayor de San Marcos, Lima, Peru; Museo de Zoología, Universidad de Concepción, Chile; Museu de Zoologia, Universidade de São Paulo, Brazil; Museu Nacional, Rio de Janeiro, Brazil.

Characters
The data matrix comprises 65 characters, 17 from genitalic (hemispermatophore) morphology and 48 from somatic morphology (Table 1; Appendix 1). Fifty-four characters were compiled from Ochoa's (2004) original data matrix, and 11 are new. Morphological terminology follows Vachon (1974) for trichobothrial nomenclature; Vachon (1952) and Prendini (2000) for pedipalp carinae; a modified version of Prendini (2000, 2003b) used by Ochoa et al. (2010) for tergite, sternite and metasomal carinae; Mattoni & Acosta (2005) for metasomal macrosetae; Ochoa (2004) and Mattoni & Acosta (2005) for hemispermatophore; Stahnke (1970) for other characters. Characters 24 and 45 were polymorphic in two taxa. Thirteen multistate characters were treated as unordered (nonadditive), except in some analyses, in which three (characters 40, 47 and 51) were ordered (additive). All characters were parsimony-informative.

Cladistic analysis
Analyses were conducted using TNT 1.1 (Goloboff et al. 2008). Heuristic search strategies were performed using traditional search with 100 random addition sequences (Wagner trees) followed by tree bisection-reconnection branch swapping (TBR), keeping up to 10 trees per replication (command sequence: 'hold 1000; mult=tbr replic 100 hold 10;'). The resulting trees were used as starting points for a round of TBR branch swapping (command: 'bbreak=TBR'). All trees found during searches were collapsed under 'rule 1' (minimum possible length is zero; Swofford & Beagle 1993; Coddington & Scharff 1994). The data matrix was analysed under equal weights (EW) and implied weights (IW) (Goloboff 1993). The sensitivity of results (sensu Wheeler 1995) was assessed by treating all

characters unordered and varying values of the concavity constant in the IW analyses (k = 1–10, 12 and 15; command: 'Piwe=N', where 'N' is the k value). Twenty-six analyses were conducted in total, 13 (one EW and 12 IW analyses) with characters 40, 47 and 51 ordered, and 13 in which these characters were unordered. Results of the sensitivity analysis were summarized by means of a 50% majority rule tree (command: 'majority=50'), whereas different optimal trees obtained from each analysis were summarized by means of strict consensus trees (command: 'nelsen'). Adjusted homoplasy (or distortion) in IW analyses, measured with a convex increasing function (Goloboff 1993, 1997), was calculated in TNT (command: 'fit'). Character optimization was conducted using accelerated transformation (ACCTRAN) (Farris 1970; Swofford & Maddison 1987, 1992) in WINCLADA 1.00.08 (Nixon 1999–2002). Unambiguous optimizations were produced using TNT (command: 'apo-').

The relative degree of support for each node (Bremer support, BS; Bremer 1994) was calculated using TNT, by searching for suboptimal trees one step longer, keeping 1000 trees per replication, until the BS was obtained for each branch (command: 'bsupport;'). Relative Bremer support (RBS), which takes into account the relative amounts of contradictory and favourable evidence (Goloboff & Farris 2001), was calculated similarly, using only trees within absolute support (command: 'bsupport];'). RBS for a group varies between 0 (completely unsupported) and 100 (entirely uncontradicted). Support values were also estimated using group frequencies under jackknifing (Jackknife support, JS; reviewed by Goloboff et al. 2003), with a probability of alteration, P = 0.36, by performing 1000 pseudoreplicates of 10 random addition sequences, each followed by TBR swapping, keeping up to 10 trees (command sequence: 'mult: noratchet repl 10 tbr hold 10; resample jak repl 1000;'). The average JS of each phylogenetic hypothesis was calculated with TNT and used as a criterion for choosing among alternative trees (Källersjö et al. 1999).

Homoplasy and synapomorphy
The consistency index (CI; Kluge & Farris 1969) and retention index (RI; Farris 1989) of the trees and characters were calculated with TNT using the macro script 'statsall.run' developed by Peterson L. Lopes (Universidade de São Paulo). The CI and RI are the simplest character statistics used to describe levels of homoplasy and synapomorphy, respectively. The average CI and RI were also calculated for some character systems, e.g., pigmentation (characters 0–5), external morphology excluding trichobothria (characters 6–21, 29–47), trichobothria (characters 22–28) and hemispermatophores (male genitalia, characters 48–64).

Results

A single most parsimonious tree (MPT) of 167 steps was obtained in most analyses under IW (k = 3–9, 10, 12 and 15, Figs 2 and 4). The sensitivity of the results of IW to different values of the concavity constant is presented in Table 3 and Fig. 5. The topology was stable in 10 of the 12 weighting regimes explored. Analyses with weaker concavity functions (i.e. those in which homoplastic characters were penalized to a lesser extent) demonstrated higher average support.

Analyses with EW and three ordered multistate characters obtained 18 MPTs (167 steps, CI = 0.479, RI = 0.813). Although some branches collapsed in the strict consensus tree (Fig. 3), the major clades were
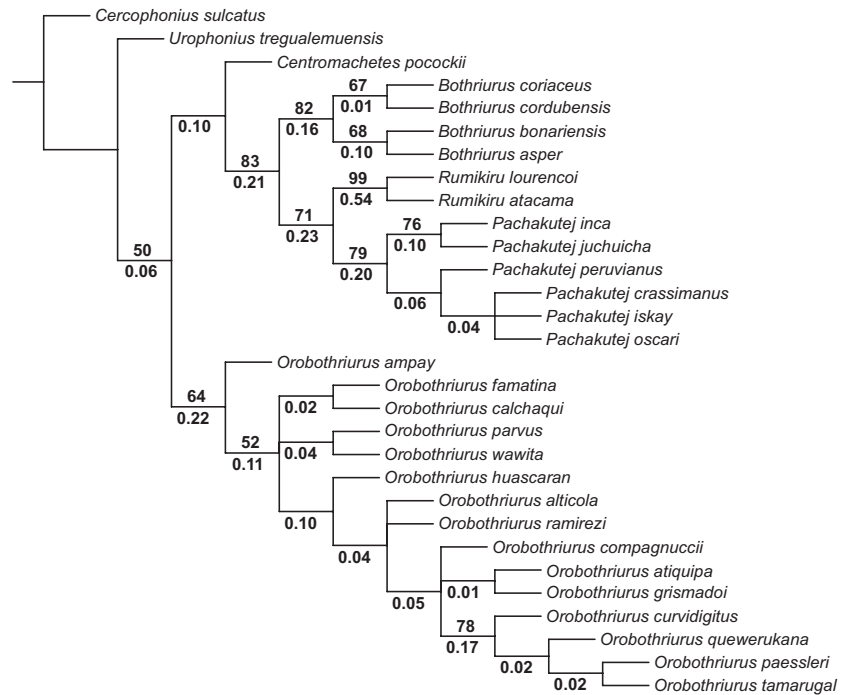
Fig. 2 Most parsimonious tree (adjusted homoplasy = 5.001, length = 167) obtained in cladistic analysis of 30 bothriurid scorpion species, including all species of *Orobothriurus* Maury, 1976, *Pachakutej* Ochoa, 2004 and *Rumikiru* Ojanguren Affilastro *et al.*, in press, under implied weights (k = 15, three characters ordered). Jackknife percentages indicated above branches. Bremer support values, in units of fit, indicated below branches. Zero length branches collapsed under 'rule 1'.

Table 3 Summary of statistical differences among most parsimonious trees (MPTs) obtained by analyses of 30 bothriurid scorpion species, including all species of *Orobothriurus* Maury, 1976, *Pachakutej* Ochoa, 2004 and *Rumikiru* Ojanguren Affilastro *et al.*, in press, with equal weights (EW) and implied weights (IW) with 12 values for the concavity constant (k)

	Length	MPTs	CI	RI	AH	Av. JS
EW	167/164	18/9	0.479/0.488	0.813/0.816	–	28.6/34.0
IW k = 1	171/168	7/7	0.468/0.476	0.804/0.807	27.78/26.03	32.9/35.8
IW k = 2	167–169/167–168	20/8	0.473–0.479/0.476–0.479	0.808–0.813/0.807–0.809	20.78/19.58	33.0/36.2
IW k = 3	167/164	1/1	0.479/0.488	0.813/0.816	16.61/15.73	32.8/36.3
IW k = 4	167/164	1/1	0.479/0.488	0.813/0.816	13.87/13.17	32.7/36.1
IW k = 5	167/164	1/1	0.479/0.488	0.813/0.816	11.92/11.34	32.7/36.1
IW k = 6	167/164	1/1	0.479/0.488	0.813/0.816	10.46/9.97	31.1/36.1
IW k = 7	167/164	1/1	0.479/0.488	0.813/0.816	9.32/8.89	32.5/35.9
IW k = 8	167/164	1/1	0.479/0.488	0.813/0.816	8.41/8.03	32.5/36.2
IW k = 9	167/164	1/1	0.479/0.488	0.813/0.816	7.66/7.32	34.3/38.0
IW k = 10	167/164	1/1	0.479/0.488	0.813/0.816	7.04/6.73	34.3/38.0
IW k = 12	167/164	1/1	0.479/0.488	0.813/0.816	6.05/5.79	34.5/**41.9**
IW k = 15	167/164	1/1	0.479/0.488	0.813/0.816	5.00/4.80	**34.7**/41.8

Values presented for analyses with three characters (40, 47 and 51) ordered (additive) or unordered (non-additive).

Length, number of steps under equal weights; MPTs, number of most parsimonious trees; CI, consistency index; RI, retention index; AH, adjusted homoplasy under concave weighting function of IW analyses; Av. JS, average support using Jackknife resampling (highest values in bold).
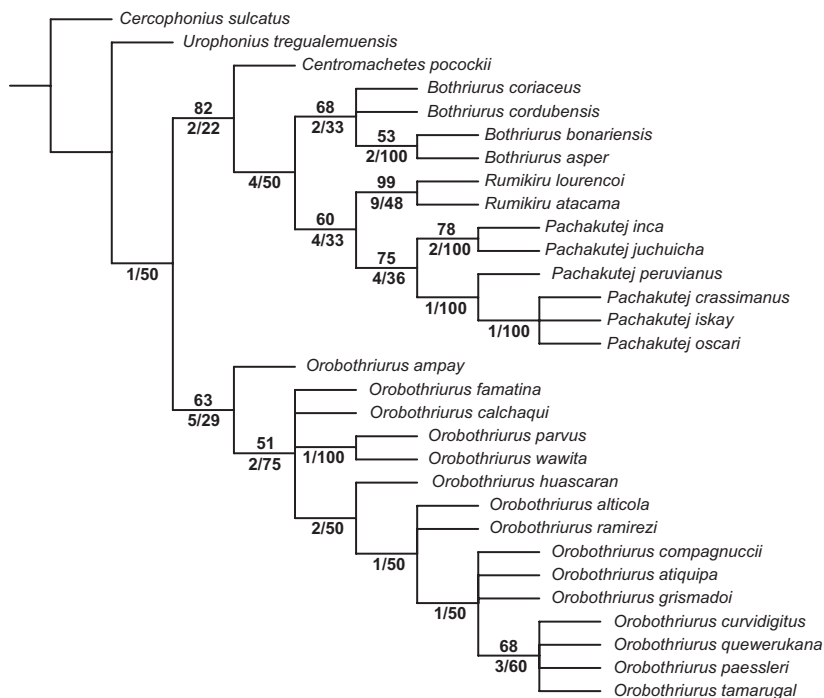
Fig. 3 Strict consensus of 18 most parsimonious trees obtained in cladistic analysis of 30 bothriurid scorpion species, including all species of *Orobothriurus* Maury, 1976, *Pachakutej*, Ochoa 2004 and *Rumikiru* Ojanguren Affilastro *et al.*, in press, under equal weights with three characters ordered (length = 167, CI = 0.479, retention index = 0.813). Jackknife percentages indicated above branches. Bremer support/relative Bremer support values indicated below branches.

maintained and were congruent with those in the topologies obtained by analyses with IW. Similar topologies were obtained when all multistate characters were unordered. A single MPT (164 steps, CI = 0.488, RI = 0.816) was obtained in most IW analyses (k = 3–9, 10, 12 and 15) and nine MPTs were obtained with EW (164 steps, CI = 0.488, RI = 0.816; Table 3).

Rumikiru was monophyletic, strongly supported by 11 unambiguous synapomorphies, BS = 9, RBS = 48, and JS = 99–100 (Figs 2–4; Appendix 2), and placed sister to *Pachakutej* in all analyses, a relationship supported by five unambiguous synapomorphies, JS = 62–84, BS = 4, and RBS = 33. *Pachakutej* was also monophyletic in all analyses and supported by five unambiguous synapomorphies, JS = 77–84, BS = 4, and RBS =36. The monophyletic group comprising *Pachakutej* and *Rumikiru* was, in turn, sister to *Bothriurus* and supported by five unambiguous synapomorphies, JS = 75–86, BS = 4 and RBS = 50.

Orobothriurus, as redefined by Ochoa *et al.* (2011), was monophyletic in all analyses and supported by six unambiguous synapomorphies and a high BS = 5 (Figs 2–5; Appendix 2). The low values of JS = 51–67 and RBS = 29 indicate some contradictory evidence for the monophyly of *Orobothriurus*, however. A monophyletic group of four *Orobothriurus* species (*O. curvidigitus*, *O. paessleri*, *O. quewerukana* and *O. tamarugal*) was well supported in all analyses by six synapomorphies, JS = 69–78, BS = 3, and RBS = 60. With the exception of this group, other relationships among the species of *Orobothriurus* were weakly supported, and not consistently recovered in all analyses (Fig. 5).

Consistency and retention indices for each character in the IW analysis with k = 15 are indicated in Fig. 5. The average CI for each character system was as follows: 0.443 (pigmentation), 0.597 (somatic morphology excluding trichobothria), 0.422 (trichobothria), and 0.752 (hemispermatophore). The average RI was as follows: 0.747 (pigmentation), 0.715 (somatic morphology excluding trichobothria), 0.808 (trichobothria), and 0.851 (hemispermatophore). The average CI and RI for all somatic characters were 0.552 and 0.733, respectively.

Discussion

New genus justified

The phylogenetic relationships retrieved in the reanalysis of *Orobothriurus* phylogeny mostly corroborated Ochoa's (2004) phylogenetic hypothesis, which was based on a smaller sample of taxa and characters. The topologies of the two studies are congruent in most respects, in spite of the different taxon samples. Both studies supported the monophyly of *Pachakutej*, its placement in a monophyletic group with *Bothriurus*, and the monophyly of *Orobothriurus*. Only a few of the relationships among species of *Orobothriurus* were incongruent.

Orobothriurus would be rendered paraphyletic by the grouping of *O. lourencoi* (and the closely related, new species from the Atacama) with *Pachakutej*, had *Orobothriurus*

Fig. 4 Single most parsimonious tree obtained in cladistic analysis of 30 bothriurid scorpion species, including all species of *Orobothriurus* Maury, 1976, *Pachakutej*, Ochoa 2004 and *Rumikiru* Ojanguren Affilastro *et al.*, in press, under implied weights (*k* = 15, length = 167, three characters ordered). Characters optimized with accelerated transformation. Homoplasious and nonhomoplasious transformations respectively indicated by white and black squares along branches, with character numbers above and character states below each. Zero length branches collapsed under 'rule 1'. Node numbers, used in synapomorphy list (Appendix 2), indicated at nodes.

not been redefined to exclude them (Ochoa *et al.* 2011). However, these two species could not have been accommodated within *Pachakutej* either (Ojanguren Affilastro *et al.*, in press). The combination of several synapomorphies with *Pachakutej*, and several unique synapomorphies, justified the need for a new genus, *Rumikiru*, to accommodate *O. lourencoi* and the new species, formal descriptions of which were provided by Ojanguren Affilastro *et al.* (in press).

Rumikiru is most closely related to *Pachakutej*, based mainly on characters of the hemispermatophore, i.e., the shared presence of one sclerotized apophysis on the internal fold of the internal lobe and a papillose fold in the basal lobe. However, species of *Pachakutej* are markedly more pigmented and possess a unique synapomorphy, a spatulate terminal process on the basal lobe of the hemispermatophore (Ochoa 2004) that is absent in *Rumikiru*. In contrast, the pedipalp carinae, especially of the femur and patella, are more pronounced in *Rumikiru* than in *Pachakutej*. The two genera also differ in the trichobothrial pattern of the pedipalp chela manus. Trichobothrium V_2 is situated in the
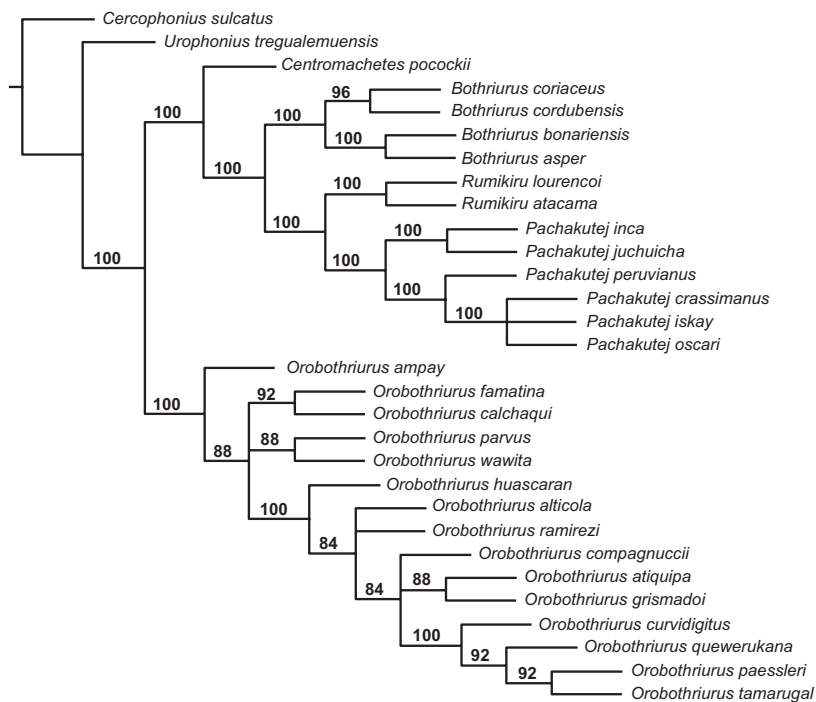
Fig. 5 Majority rule (50%) consensus of trees obtained from 26 cladistic analyses of 30 bothriurid scorpion species, including all species of *Orobothriurus* Maury, 1976, *Pachakutej*, Ochoa 2004 and *Rumikiru* Ojanguren Affilastro *et al.*, in press, under equal and implied weights, with three characters ordered or unordered. Nodes with <100% collapsed in strict consensus.

same axis as V_1 and V_3 in *Rumikiru* but not in the same axis, forming an angle <180°, in *Pachakutej*, and *Db* is equidistant from *Dt* and Eb_3 in *Rumikiru* but close to *Dt* in *Pachakutej*. Finally, the ventrosubmedian carinae of metasomal segment V are subparallel and situated close to the ventrolateral carinae in *Rumikiru*, whereas the ventrosubmedian carinae are slightly inclined towards the external margin in the posterior third of the segment, or absent, in *Pachakutej*.

Also noteworthy is the marked difference in habitat and geographical distribution of *Rumikiru*, both species of which occur in the Atacama Desert (Absolute and Coastal Desert subregion, as defined by Gajardo 1995) of Chile, compared with species of *Pachakutej*, which occur in more humid habitats, in inter-Andean valleys and montane rainforests on the eastern slopes of the Andes in Peru (Ochoa 2004; Fig. 1).

The two species of *Rumikiru* differ from *Orobothriurus*, *Pachakutej* and all other bothriurid genera in several respects (Ojanguren Affilastro *et al.* in press). Both species display an enlarged basal denticle in the median denticle row of the pedipalp chela movable finger, which is approximately three times larger than, and replaces the first five or six median denticles. Such a hypertrophied basal denticle is unique among bothriurids. An enlarged basal denticle on the movable finger is exhibited by some species, e.g., *Brachistosternus ehrenbergii* (Gervais, 1841), but replaces no more than two or three denticles in these cases (Ochoa & Ojanguren Affilastro 2007). The submedial position of the apophysis on the internal surface of the pedipalp chela manus of adult males is also unique to *Rumikiru*. The apophysis is situated in the distal third of the surface in all other bothriurid taxa in which it occurs, and secondary sexual structures are absent from the pedipalp chela manus of basal bothriurids, e.g., *Lisposoma* Lawrence, 1928 and *Thestylus* Simon, 1880 (Prendini 2000, 2003a). The shape of the apophysis and position of trichobothium *ib* distal to *it* are also unique to *Rumikiru*. *Rumikiru* is further characterized by a twist in the medial to distal third of the dentate margin of the pedipalp chela fingers, which abruptly alters the orientation of the median denticle row (more conspicuously on the movable finger), a character observed only in the adult male that is absent in other bothriurid genera. Additionally, the carapace of *Rumikiru* is dorsoventrally compressed, unlike most other bothriurids, in which it is more convex.

Orobothriurus phylogeny

This study increased the number of synapomorphies for *Orobothriurus* from what was previously proposed (Ochoa 2004). Notable among these synapomorphies is the shape of the female genital opercula, which are distinctly elongated posteriorly in *Orobothriurus*. This character exhibits a parallelism in *Pachakutej* and *Rumikiru*, but is distinctly different from that observed among all other bothriurid taxa, in which the genital opercula are rhomboid in shape. The two distinct opercular shapes may be associated with differences in the mechanism of sperm transfer. During

sperm transfer, the posterior tips of the female opercula fit into the space between the frontal crest and the lamina of the male spermatophore (Peretti 2003, 2010), hence, the elongated female opercula may be an adaptation to the enlarged frontal crest of the male hemispermatophore in *Orobothriurus*. This may also be true in *Pachakutej*, which possess an enlarged frontal crest and elongated genital opercula. However, nothing is known about the mechanism of sperm transfer in these genera.

Acosta & Ochoa (2001) proposed two subgroups within the former *alticola* group of *Orobothriurus*, to which the genus was later restricted (Ochoa 2004). The first subgroup comprised species in which the apex of the hemispermatophore is elongated and the frontal crest reduced (*O. ampay*, *O. parvus* and *O. wawita*), whereas the second subgroup included species in which the apex is reduced and the frontal crest well developed (all other species of the genus). The reanalyses of *Orobothriurus* phylogeny do not support this proposal, however. Only the second subgroup was weakly supported by four unambiguous synapomorphies (no JS support; BS = 2). *Orobothriurus ampay* was placed basal to all other species of *Orobothriurus*. This species possesses several distinctive characters compared with the other species, e.g., a dark pigmentation pattern, and pronounced ventrosubmedian carinae on the metasomal segments, which appear to be plesiomorphic for the genus.

Four species, characterized by a weak median projection (epistome) of the anterior margin of the carapace and a distinctly curved movable finger on the pedipalp chela of the adult male (*O. curvidigitus*, *O. paessleri*, *O. quewerukana* and *O. tamarugal*), formed a well-supported group in all analyses. These species also occur in fairly close geographical proximity in southern Peru and northern Chile (Fig. 1). *Orobothriurus parvus* appears to be related to *O. wawita*, and *O. calchaqui* to *O. famatina*. However, these groups and several others within the genus were weakly supported (Figs 2–4) and some, e.g., the putative relationship between *O. atiquipa* from the coast of southern Peru and *O. grismadoi* from an isolated extinct volcano, over 2000 m in Mendoza, Argentina, cannot be easily explained based on the known distributions. The placement of *O. atiquipa* among several species from Argentina may be an artefact of missing entries in five characters of the female, which is unknown in this species. The general weakness of these internal nodes suggests the need for additional characters, e.g., DNA sequences.

Andean biogeography
Orobothriurus displays an Andean pattern of distribution, most species occurring at high altitudes (over 2000–2500 m to a maximum altitude record of 4910 m) from central Peru to Argentina (Fig. 1). Only two species, *O. atiquipa* and *O. paessleri*, occur at lower altitudes (between 700 and 950 m), in lomas formations, endemic plant communities along the Pacific desert coastline, usually situated on hills, where ocean fog provides sufficient moisture for the development of vegetation (Péfaur 1981; Dillon 2005), in southern Peru (Ochoa 2004, 2005). One species, *O. tamarugal*, inhabits the isolated forests of *Prosopis tamarugo* Phil. (Fabaceae) in northern Chile. The genus may have originated in southern Peru, as suggested by the basal placement in the phylogeny of the southern Peruvian species *O. ampay*, *O. parvus* and *O. wawita*.

All *Orobothriurus* species appear to have limited vagility and very restricted distributional ranges (Ochoa *et al.* 2011). The presence of different species on both sides of the Andes suggests that the origin of the genus predates the main uplift of this mountain range. Andean orogeny strongly influenced the distribution of organisms (Vuilleumier 1971; Antonelli *et al.* 2009; Graham 2009) and promoted significant changes in the climate of central and southern South America (Vuilleumier 1971; Strecker *et al.* 2007; Insel *et al.* 2010), perhaps contributing to speciation processes in *Orobothriurus*.

High Andean *Orobothriurus* species can cope with harsh environments, where they are exposed to very low temperatures when active at night, and can survive the winter at high altitudes where heavy snowfalls are common. Therefore, the absence of *Orobothriurus* from the Altiplano and Puna plateaus (Fig. 1), which have been well surveyed for scorpions by members of our research group, and where other scorpion taxa (e.g. the bothriurid genus, *Brachistosternus* Pocock, 1893) have been recorded, cannot be explained by the harsh conditions. One possible factor could be the recent (10 million years to the present) and sustained vulcanism that affected the central and southern Andes where volcanoes are abundant (Trumbull *et al.* 2006; Fig. 1), thereby altering the chemical and/or physical composition of the soil, which is fundamental for the survival of fossorial and lapidicolous scorpions (Prendini 2001). The potentially lethal effects of vulcanism, perhaps combined with extensive flooding in the northern and central Altiplano (Fornari *et al.* 2001), and the low vagility of *Orobothriurus* species, might explain their absence. Indeed, no scorpions have been recorded from the central area of the Altiplano, where both vulcanism and flooding occurred (Acosta & Ochoa 2002; Ojanguren Affilastro 2003b).

Other high-altitude scorpions with greater vagility and wider distributional ranges do occur in the Altiplano, however (Ochoa & Acosta 2002; Ojanguren Affilastro 2002, 2003b, 2004). Four species of *Brachistosternus* have been recorded there, a small number compared with adjacent areas of Chile and Peru (Ojanguren Affilastro 2003b;

Ochoa 2005; Ojanguren Affilastro & Mattoni 2006; Ojanguren Affilastro & Scioscia 2007; Ojanguren Affilastro *et al.* 2007). *Brachistosternus intermedius* Lönnberg, 1902 occupies the southern and eastern parts of the Altiplano (Ojanguren Affilastro 2004). *Brachistosternus titicaca* Ochoa & Acosta, 2002 occurs at the northern limit of the Altiplano, around Lake Titicaca (Ochoa & Acosta 2002). *Brachistosternus galianoae* Ojanguren Affilastro, 2002 is restricted to the slopes of Sajama Volcano in Bolivia, at very high altitudes above 4300 m. *Brachistosternus piacentinii* Ojanguren Affilastro, 2003 also occurs on the slopes of Sajama Volcano, and along the western margin of the Andes in Chile, in a region that does not belong to the Altiplano (Ojanguren Affilastro & Ramírez 2009). All four *Brachistosternus* species are related to other high-altitude species occurring outside the Altiplano (Ojanguren Affilastro & Ramírez 2009), suggesting that speciation on or near the Altiplano followed recent colonization events from the Andes.

The central Andes (Peruvian and Bolivian Altiplano) experienced a surface uplift of 2300–3400 m during the past 10.7 million years (since the Late Miocene), termed the 'Quechua' phase (Sébrier *et al.* 1988; Gregory-Wodzicki 2000; González & Pfiffner 2012). This phase was accompanied by intensified vulcanism (Trumbull *et al.* 2006). The main diversification of *Orobothriurus* probably occurred much earlier (ca. 25 million years ago), when the Andes were about 3000 m high, and volcanic events less frequent (Gregory-Wodzicki 2000; Trumbull *et al.* 2006). The species from the lomas formations could have evolved much more recently, perhaps during the Pliocene (5–1.8 million years). Many plants and animals endemic to lomas formations have close relatives in the Andes and are thought to have evolved from dispersal and subsequent isolation associated with recent expansions and contractions of the Andean vegetation (Herrera 1930; Dillon 2005; Palma *et al.* 2005). Molecular-based geographical studies are needed to confirm whether these *Orobothriurus* species evolved in a similar manner.

Homoplasy in genitalic vs. somatic characters
It has been argued that the genitalic characters of arthropods evolve relatively rapidly, because of strong sexual selection, and have low phylogenetic inertia (Arnqvist & Rowe 2002; Eberhard 2004), implying that genitalic characters could be very homoplastic and therefore misleading, or at least uninformative, in phylogenetic studies. Song & Bucheli (2010) demonstrated empirically, using 41 published data sets, that genitalic and somatic characters have similar levels of homoplasy in insects, but comparisons among arachnids are lacking.

We compared the levels of homoplasy among genitalic and somatic characters in bothriurid scorpions, which exhibit some of the greatest diversity in male genitalia (i.e. hemispermatophore) among scorpions (Maury 1980; Ojanguren Affilastro 2005; Peretti 2010). Characters from the male genitalia showed higher CI and RI indices than somatic characters, suggesting that genitalic characters of arachnids are at least as informative as somatic characters, if not more so. As concluded by Song & Bucheli (2010), genitalic characters contain a strong phylogenetic signal, despite a high level of morphological variation.

We agree further with Song & Bucheli (2010) that the composite nature of genitalia renders genitalic character systems phylogenetically informative at multiple levels in the taxonomic hierarchy. Different levels of information are constrained by the functional morphology of the structures in question. In scorpions, which inseminate females by means of a spermatophore attached to the substrate, structures that perform a similar, mechanically constrained function, e.g., the lamina and the basal portion, are probably under strong stabilizing selection (Peretti 2010). These parts of the spermatophore (and its component hemispermatophores) would therefore be expected to exhibit limited variation among closely related taxa, instead providing phylogenetic information for deeper nodes, and that is indeed the case. In contrast, structures that make contact with the female gonopore and genital opercula, e.g., the lobe region and surrounding areas of the spermatophore, are probably under strong sexual selection (Eberhard 1985; Peretti 2010), and would therefore be expected to provide phylogenetic information among closely related taxa and, indeed, it is these structures in which the greatest morphological diversity is observed (Peretti 2003).

It is also interesting to note that the least homoplastic somatic characters pertain mostly to the chelicerae and pedipalps (characters 9–21, Fig. 6), used by male scorpions to grasp and secure females during mating (Polis & Sissom 1990; Carrera *et al.* 2009). Both appendages may therefore also be under strong stabilizing selection and consequently exhibit less homoplasy than other parts of the somatic morphology.
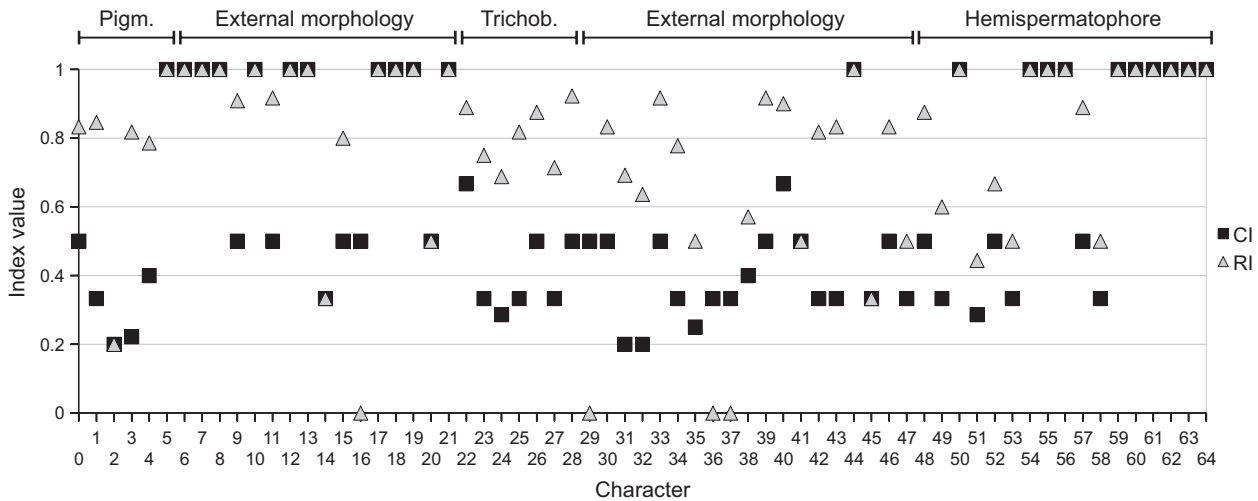
Acknowledgements

Fig. 6 Consistency indices (CI, black squares) and retention indices (RI, grey triangles) of characters used in cladistic analysis of 30 bothriurid scorpion species, including all species of *Orobothriurus* Maury, 1976, *Pachakutej* Ochoa, 2004 and *Rumikiru* Ojanguren Affilastro *et al.*, in press, calculated on most parsimonious tree obtained under implied weights ($k = 15$). Abbreviations: Pigm., pigmentation; Trichob., trichobothria.

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Appendix 1
Character list

List of 65 characters scored for cladistic analysis of 30 bothriurid scorpion species, including all species of *Orobothriurus* Maury, 1976, *Pachakutej* Ochoa, 2004 and *Rumikiru* Ojanguren Affilastro *et al.*, in press. Data matrix in Table 1. Character states are scored 0–3, unknown (?) and inapplicable (-). Morphological terminology follows Vachon (1974) for trichobothrial nomenclature; Vachon

(1952) and Prendini (2000) for pedipalp carinae, abbreviated as follows: internomedian (IM), dorsointernal (DI), dorsomedian (DM), dorsoexternal (DE), externomedian (EM), ventroexternal (VE), ventromedian (VM), ventrointernal (VI), dorsal patellar process (DPP), ventral patellar process (VPP), digital (D), dorsal secondary (DS), dorsomarginal (DMA), external (E); a modified version of Prendini (2000, 2003b) used by Ochoa *et al.* (2010) for tergite, sternite and metasomal carinae, abbreviated as follows: dorsolateral (DL), lateral supramedian (LSM), lateral median (LM), lateral inframedian (LIM), ventrolateral (VL), ventrosubmedian (VSM), ventromedian (VM); Mattoni & Acosta (2005) for metasomal macrosetae; Ochoa (2004) and Mattoni & Acosta (2005) for hemispermatophore; Stahnke (1970) for other characters. Characters from previous analyses that correspond partially or entirely to those in the present matrix are as follows: O2004 = Ochoa (2004); OA&R2009 = Ojanguren Afilastro & Ramírez (2009); P2003 = Prendini (2003a).

Pigmentation pattern

0. Tergites I–IV, pigmentation: entirely pigmented (0); paired spots sublaterally, unpigmented area medially (1) [O2004: 0; OA&R2009: 1].

1. Tergite VII, pigmentation: entirely pigmented (0); paired spots sublaterally, unpigmented area medially (1); inapplicable (-).

2. Metasomal segments II and III, dorsal surfaces, pigmentation: absent, unpigmented (0); subtriangular spot medially, may be divided by unpigmented line medially (1).

3. Metasomal segments I–III, dorsal surfaces, pigmentation along DL carinae: absent, unpigmented (0); reticulate lines (1).

4. Metasomal segments IV and V, ventral surfaces, VM stripe: contiguous with VL stripe posteriorly (0); not contiguous with VL stripe posteriorly (1); absent (2). [OA&R2009: 5].

5. Telson vesicle, ventral and lateral surfaces, colouration ($\male$): similar to $\female$, pigmented (0); different from $\female$, unpigmented, with glandular, light-yellow colouration (1).

Carapace

6. Anterior margin, shape: sublinear or with shallow median notch (0); with weak median projection (epistome) (1). [OA&R2009: 7].

7. Anteromedian longitudinal sulcus, length ($\male$): complete (0); vestigial (1). [P2003: 4; O2004: 1; OA&R2009: 8].

Chelicera

8. Movable finger, subdistal teeth, number: one (0); two (1). [P2003: 9; O2004: 2; OA&R2009: 6].

Pedipalps

9. Femur, length ($\male$): greater than three times width (0); <3 times width (1). [O2004: 3; OA&R2009: 75].

10. Femur, dorsal surface, shape: slightly convex, DE and DI carinae situated in different axes (0); flat, DE and DI carinae situated in same axis (1). [O2004: 4].

11. Chela, shape: similar in both sexes or narrower (length/width ratio of pedipalp chela smaller) in $\male$ than $\female$ (0); more robust (length/width ratio of pedipalp chela greater) in $\male$ than $\female$ (1). [O2004: 5; OA&R2009: 60].

12. Chela manus, external surfaces, shape ($\male$): prismatic (0) flat (1). [O2004: 6].

13. Chela manus, surfaces adjacent to DMA carinae, shape ($\male$): D-DMA-DI carinae forming angle >90° (0); D-DMA-DI carinae forming angle <90°. [O2004: 7].

14. Chela manus, DMA, DI and VM carinae, granulation ($\male$): absent, smooth (0); present, finely and densely granular (1).

15. Chela movable finger, shape ($\male$): straight (0); curved (1). [O2004: 8].

16. Chela manus, secondary sexual apophysis ($\male$): present (0); absent (1). [O2004: 9].

17. Chela manus, secondary sexual apophysis, shape ($\male$): conical (0); spiniform (1); lobate (2); conical-truncate (3); horn-like (4); inapplicable (-). [P2003: 25; O2004: 10; OA&R2009: 61].

18. Chela manus, secondary sexual apophysis, position ($\male$): close to base of fixed finger, distal to trichobothrium *ib* (0); in distal third of manus, proximal to trichobothrium *ib* (1).

19. Chela fixed finger, group of granules at base ($\male$): absent (0); present (1). [P2003: 26; O2004: 11; OA&R2009: 62].

20. Chela fingers, median denticle rows, number: single row (0); multiple rows (1). [P2003: 27; O2004: 12; OA&R2009: 64].

21. Chela movable finger, median denticle row, relative denticle size: all denticles equal in size (0); basal denticle greatly enlarged, approximately five times larger than other denticles (1).

Trichobothria

22. Chela manus, trichobothrium *ib*, position ($\male$): situated at base of ventral side of secondary sexual apophysis, visible in ventral view (0); situated at base of internal side of apophysis, not visible in ventral view (1); situated more distal to apophysis, visible in ventral view (2). [O2004: 13].

23. Chela manus, trichobothrium *Esb*, position: intermediate between Eb_1 and Eb_2 (0); dorsal to Eb_2 (1); intermediate between Eb_2 and Eb_3 (2). [O2004: 14; OA&R2009: 53].

24. Chela manus, trichobothrium Et_3, position: distal to *Est* (0); in same axis as *Est* (1); proximal to *Est* (2). [O2004: 15; OA&R2009: 54].

25. Chela manus, trichobothrium V_2, position: forming obtuse angle (<180°) with V_1 and V_3 (0); in same axis as V_1 and V_3 (1). [O2004: 16].

26. Chela fixed finger, trichobothrium *dst*, position: in same axis as *est* (0); proximal to *est* (1). [O2004: 17].

27. Chela manus, trichobothrium *Db*, position: close to *Dt* (0); equidistant between *Dt* and Eb_3 (1). [O2004: 18].

28. Chela manus, trichobothrium Et_5, position: forming acute angle (<90°) with *eb* and Et_4 (0); forming obtuse angle (<180°) with *eb* and Et_4 (1). [O2004: 19].

Legs
29. Legs III and IV, telotarsi, ventrosubmedian spiniform macrosetae, number of pairs: three (0); more than five (1). [O2004: 20; OA&R2009: 39].

Genital operculum
30. Genital sclerites, shape (♀): rhomboid (0); posteriorly elongated, anterior edge slightly rounded (1).

Sternites and Metasoma
31. Sternite VII, carinae: absent (0); VL and/or VSM carinae present (1). [O2004: 21; OA&R2009: 81].

32. Metasomal segment I, VSM carinae (♀): absent (0); present (1). [O2004: 22; OA&R2009: 91].

33. Metasomal segment II, VSM carinae (♀): present (0); absent (1). [O2004: 23; OA&R2009: 92].

34. Metasomal segment II, VSM carinae (♂): present (0); absent (1). [O2004: 24].

35. Metasomal segment III, VSM carinae (♀): present (0); absent (1). [O2004: 25].

36. Metasomal segment IV, VSM carinae (♀): present (0); absent (1). [O2004: 26; OA&R2009: 97].

37. Metasomal segment IV, VSM carinae (♂): present (0); absent (1). [O2004: 27].

38. Metasomal segment V, VL carinae (♂): present in more than half of segment (0); restricted to posterior third (1); absent or reduced to few granules posteriorly (2). [O2004: 28; OA&R2009: 104].

39. Metasomal segment V, VL and VSM carinae, relative orientation: well separated, diverging (0); adjacent, subparallel (1); inapplicable (-). [O2004: 29].

40. Metasomal segment V, VSM carinae, orientation in posterior third: subparallel, not diverging (0); diverging slightly (1), diverging strongly, forming arc (2). [P2003: 82; O2004: 30; OA&R2009: 101; additive].

41. Metasomal segment V, VM carina: present, well developed (0), vestigial or absent (1). [O2004: 31].

42. Metasomal segment III, length (♂): less than or equal to width (0); greater than width (1). [O2004: 32; OA&R2009: 105].

43. Metasomal segment V, length (♂): less than or equal to twice width (0); greater than twice width (1). [O2004: 33; OA&R2009: 106].

Macrosetae
44. Pedipalp femur, dorsal macroseta (M_1) situated near *d* trichobothrium: present (0), absent (1). [O2004: 34].

45. Metasomal segment I, VSM macrosetae, number of pairs: two (0); three (1). [O2004: 35; OA&R2009: 83].

46. Metasomal segment III, VSM setae, number of pairs: two (0); three (1). [O2004: 36].

47. Metasomal segment IV, VSM setae, number of pairs: two (0); three (1); four (2). [O2004: 37; OA&R2009: 87; additive].

Hemispermatophore
48. Apex of lamina, shape of distal margin: subtriangular (0); rounded or subquadrangular (1). [O2004: 38].

49. Apex of lamina, length: greater than length of frontal crest (0); less than length of frontal crest (1); inapplicable (-).

50. Distal crest, shape: curved with respect to ventral border (0); parallel to ventral border (1); straight and diagonal to ventral border (2). [O2004: 40].

51. Semicircular depression at base of lamina, development: well developed, extended to basal portion (0); slightly developed (1); obsolete (2). [O2004: 41; additive].

52. Posterodistal fold: absent (0); present (1). [O2004: 42].

53. Frontal crest: absent (0); present (1). [O2004: 43; OA&R2009: 35].

54. Frontal crest, incision in distal border: absent (0); present (1); inapplicable (-). [O2004: 44].

55. Frontal crest, shape: undivided, with continuous, smooth borders, without undulations (0); divided in two parts, proximal oblique part with two lateral folds, distal part straight usually parallel to ventral margin of lamina, with lateral projections on each side (1); divided in two parts, distal part sinuous with strong undulation in dorsal border (2).; inapplicable (-). [O2004: 45].

56. Frontal crest, lateral projections of distal part, shape: reduced, length of projections less than length of proximal oblique part (0); elongated, length of distal part greater than proximal oblique part (1); inapplicable (-). [O2004: 47].

57. Capsule, internal lobe, development: similarly developed to external lobe (0); considerably more developed than external lobe (1). [O2004: 49].

58. Capsule, internal lobe, spiniform apophysis on external surface: absent (0); present (1). [O2004: 50].

59. Capsule, internal lobe, sclerotized apophysis on internal fold: absent (0); present (1). [O2004: 51].

60. Capsule, basal lobe, tortuous stem, subdistal spoon-like dilation ending in terminal process: absent (0); present (1). [O2004: 52].

61. Capsule, basal lobe, subdistal dilation, shape: widened (0); oval (1); elongated (2); inapplicable (-). [O2004: 53].

62. Capsule, basal lobe, terminal process, shape: short (0); elongated (1); inapplicable (-). [O2004: 54].

63. Capsule, basal lobe, spatulate terminal process: absent (0); present (1). [O2004: 55].

64. Capsule, basal lobe, papillose fold: absent (0); present (1). [O2004: 56].

Appendix 2
Synapomorphy list
List of unambiguous synapomorphies for the preferred hypothesis of relationships among 30 bothriurid scorpion species, including all species of *Orobothriurus* Maury 1976, *Pachakutej* Ochoa 2004 and *Rumikiru* Ojanguren Affilastro *et al.* in press: analysis under IW, *k* = 15, with three ordered multistate characters. Node numbers refer to Fig. 4. Character numbers refer to Appendix 1. Character transformations are denoted by listing ancestral and derived states separated by ' → '.

Urophonius tregualemuensis: Char. 0: 0 → 2; 1: 0 → 1; 2: 1 → 0; 4: 0 → 1; 29: 0 → 1; 35: 0 → 1

Centromachetes pocockii: Char. 14: 0 → 1; 23: 0 → 2; 24: 1 → 0; 27: 1 → 0; 29: 0 → 1

Bothriurus coriaceus: Char. 2: 1 → 0; 3: 1 → 0

Bothriurus cordubensis: Char. 0: 0 → 1

Bothriurus bonariensis: Char. 48: 0 → 1; 51: 1 → 2; 55: 0 → 2

Bothriurus asper: Char. 0: 0 → 1; 24: 1 → 0; 47: 0 → 1; 51: 1 → 0

Pachakutej inca: Char. 9: 1 → 0

Pachakutej juchuicha: Char. 4: 0 → 1

Pachakutej peruvianus: Char. 32: 0 → 1

Pachakutej oscari: Char. 51: 2 → 1

Orobothriurus ampay: Char. 16: 0 → 1; 45: 0 → 1

Orobothriurus famatina: Char. 2: 1 → 0

Orobothriurus calchaqui: Char. 24: 1 → 2

Orobothriurus parvus: Char. 61: 1 → 0

Orobothriurus wawita: Char. 4: 1 → 2; 16: 0 → 1; 31: 1 → 0; 34: 0 → 1; 35: 0 → 1; 38: 0 → 2; 41: 0 → 1

Orobothriurus huascaran: Char. 23: 1 → 2; 27: 1 → 0; 47: 1 → 2

Orobothriurus compagnuccii: Char. 49: 1 → 0

Orobothriurus atiquipa: Char. 31: 1 → 0; 47: 1 → 2

Orobothriurus grismadoi: Char. 1: 1 → 0

Orobothriurus curvidigitus: Char. 23: 1 → 2

Orobothriurus paessleri: Char. 14: 1 → 0; 23: 1 → 2; 45: 0 → 1

Orobothriurus tamarugal: Char. 2: 1 → 0; 4: 1 → 2; 24: 2 → 1; 32: 0 → 1

Node 2: Char. 19: 1 → 0; 52: 1 → 0

Node 3: Char. 9: 0 → 1; 10: 1 → 0; 11: 0 → 1; 43: 1 → 0; 46: 1 → 0; 47: 1 → 0

Node 4: Char. 7: 0 → 1; 33: 0 → 1; 34: 0 → 1; 35: 0 → 1; 50: 1 → 0

Node 5: Char. 12: 0 → 1; 13: 0 → 1; 22: 0 → 1; 40: 0 → 2; 44: 1 → 0

Node 6: Char. 52: 0 → 1

Node 7: Char. 8: 1 → 0; 23: 0 → 2

Node 8: Char. 30: 0 → 1; 48: 0 → 1; 51: 1 → 2; 59: 0 → 1; 64: 0 → 1

Node 9: Char. 5: 0 → 1; 15: 0 → 1; 17: 0 → 4; 18: 0 → 1; 21: 0 → 1; 22: 0 → 2; 25: 0 → 1; 39: 0 → 1; 42: 0 → 1; 43: 0 → 1; 50: 0 → 2

Node 10: Char. 24: 1 → 0; 27: 1 → 0; 46: 0 → 1; 47: 0 → 1; 63: 0 → 1

Node 11: Char. 38: 0 → 2; 41: 0 → 1

Node 12: Char. 40: 0 → 1

Node 13: Char. 38: 0 → 1

Node 14: Char. 22: 0 → 1; 23: 0 → 1; 30: 0 → 1; 39: 0 → 1; 51: 01 → 2; 60: 0 → 1

Node 15: Char. 0: 0 → 1; 1: 0 → 1; 4: 0 → 1

Node 16: Char. 3: 1 → 0; 61: 1 → 2

Node 17: Char. 2: 1 → 0; 47: 1 → 2

Node 18: Char. 24: 1 → 2; 49: 0 → 1; 56: 0 → 1; 62: 0 → 1

Node 19: Char. 3: 1 → 0; 35: 0 → 1

Node 20: Char. 34: 0 → 1

Node 21: Char. 45: 0 → 1

Node 22: Char. 6: 0 → 1; 14: 0 → 1; 15: 0 → 1; 31: 1 → 0; 32: 1 → 0; 33: 0 → 1

Node 23: Char. 11: 0 → 1; 49: 1 → 0

Node 24: Char. 43: 1 → 0

Supporting Information
Additional Supporting Information may be found in the online version of this article:

Appendix S1. Material examined.

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