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Deep soil floatation in Chile reveals diverse and mainly nameless fauna of endogean beetles (Coleoptera)

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Abstract

We present the findings of a field survey conducted to study deep soil (=endogean) beetles in Chile. We have analysed 50 soil samples, each weighing about 120 kg, collected from 15 central Chilean forested localities along or near the Coastal Range between Valdivia and Santiago. We illustrate all 2,037 adult specimens of Chilean endogean beetles. These specimens include representatives of 11 families: Carabidae, Corylophidae, Curculionidae (subfamilies Cossoninae, Cyclominae, and Raymondionyminae), an unidentified family of Elateroidea, as well as Eupsilobiidae, Leiodidae, Ptiliiidae, Scarabaeidae, Staphylinidae (subfamilies Aleocharinae, Euaesthetinae, Leptotyphlinae, Osoriinae, Pselaphinae, Scydmaeninae, and Staphylininae), Tenebrionidae, and Zopheridae. The most abundant groups are Leptotyphlinae, Leiodidae and Pselaphinae accounting for 45%, 17%, and 17% of all specimens, respectively. The number of adult endogean beetles per soil sample varied between 1 and 150, with an average of 41 specimens (equivalent to an average of 0.34 specimens per kilogram of soil). For each of the 15 sampled localities, specimens were classified to morphospecies, and for each morphospecies we provide the most accurate identification currently possible, considering that most of the species detected are either unnamed or belong to taxonomically challenging groups. In addition, for each morphospecies and locality at least one specimen was selected to be individually vouchered, DNA extracted, and sequenced for the reference barcode DNA fragment in Metazoa. A total of 190 specimens were selected, obtaining barcode sequences for 187 (98.4% success rate) which are provided in an open access DNA barcode library at BOLD Systems (dx.doi.org/10.5883/DS-VGDS28). Obtained sequences were clustered by BOLD Systems in 119 BINs, all corresponding to unique BINs exclusively formed by the newly generated sequences. In addition, when compared to NCBI databases, sequences yielded no matches with a similarity higher than 97%. The comparison with both, BOLD Systems and NCBI databases, supports that none of the deep soil beetle species here collected in Chile has been previously DNA barcoded. We conclude that the deep-soil beetle fauna of Chile is much richer than previously known and mainly unnamed at the species level.

Keywords Coleoptera | beetle | endogean | deep soil | Chile

1. Introduction

The soil is a highly heterogeneous environment that varies across space according to multiple biological, physical, and climatic parameters. Different soil compartments harbour different soil fauna communities. Animals obligatorily inhabiting deep soil layers characterised by a constantly dark and confined environment, are named

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'endogean' (Coiffait 1958, Giachino & Vailati 2010). Beetles have recurrently colonised the deep soil, with endogean species belonging to distantly related lineages united by a shared set of biological and morphological adaptations to the life in the soil. Such adaptations include partial or complete reduction of eyes and hind wings, as well as relatively small and uniformly pale bodies.

The way of life and morphological characteristics of endogean beetles suggest limited dispersal capacity, which agrees with a very small spatial scale for geographic intraspecific differentiation and speciation (Andújar et al. 2015, Arribas et al. 2016). The low dispersal capacity also explains the lack of endogean beetles in areas that were glaciated during the Pliocene-Pleistocene climatic cycles (e.g., most of Canada, or Central and Northern Europe). After permafrost erased endogean communities, deep soil beetles had no time to recolonise recently deglaciated areas. The few endogean beetles reported in the UK and other North European countries are likely human introductions (e.g., Olberg & Olsen 2009, Versluijs et al. 2013, Drumont et al. 2020). On the other hand, the low capacity for active dispersal is not at odds with the nearly global distribution of some of these taxa, such as Anillini ground beetles (Andújar et al. 2016) or Leptotyphlinae rove beetles (Herman 2001b). The latter is likely due to the ancient (pre-Cenozioc) age of these endogean lineages predating plate tectonic events and/or rare cases of passive long distance (=transoceanic) dispersal (Andújar et al. 2016).

Due to their small size and cryptic habitat, endogean beetles are rarely encountered. In this respect endogean beetles are terrestrial analogues to the inhabitants of the oceanic abyss, the latter also rarely seen and inadequately known (Jamieson & Weston 2023). In both cases, dedicated and labour-intensive sampling, if efficiently applied, is likely to result in new significant discoveries. Examples of such deep soil beetle surveys are not numerous and are mainly limited to southern Europe, e.g., Fancello et al. (2009) for Italy and Bekchiev & Guéorguiev (2014) for Bulgaria. These and other studies indicate the existence of a great endemic diversity of endogean beetles within the Mediterranean region, a conclusion that likely can be extrapolated to other regions with the Mediterranean type of climate but that requires confirmation. This hypothesis agrees with the recent discovery of a diverse subterranean arthropod fauna in Western Australia (Guzik et al. 2011), including stygobiont Dytiscidae (Austin et al. 2023) and at least some endogean Carabidae (Zuphiini: Baehr 2014, Anillini: Giachino et al. 2021) and Curculionidae (Guzik et al. 2011).

The Mediterranean climate is found, in addition to the lands adjacent to the Mediterranean Sea and in South/Western Australia, on the Pacific side of the USA, in South Africa, and in Chile. For at least one and half centuries Chile is known to harbour minute eyeless and presumably deep soil beetles (Carabidae: the anilline genus *Nothanillus* Jeannel, 1962 established based on endogean specimens collected by Philibert Germain, the latter living between 1827 and 1913; Cryptophagidae: *Amydropa anophthalma* Reitter, 1877; Leschen 1996). Sampling conducted in Chile in the 60's and 70's of the 20s century by Tomás Cekalovic (Concepción, Chile; see Discussion on his record of an eyeless raymondionymine weevil) and Francisco Sáiz (Valparaíso, Chile, e.g., Sáiz 1973, 1974) detected a variety of new endogean species and genera. More recently, soil sampling efforts of two entomological couples, Stewart B. Peck and Jarmila Kukalová-Peck (Ottawa, Canada), as well as Alfred F. Newton and Margaret K. Thayer (Chicago, USA), rekindled interest in the endogean beetle fauna of the country and resulted in new discoveries (e.g., Newton 1985, Stebnicka & Skelley 2003). In all these instances, however, deep soil sampling did not form the single focal point of the fieldwork, which likely permitted at least some lineages of endogean Chilean beetles to escape detection. To address this knowledge gap, we performed a Chilean endogean beetle field survey specifically designed to sample and understand the diversity of these obscure organisms. This paper documents our methods and reports our results.

2. Material and Methods

2.1 Temporal and geographical settings

Fieldwork was performed in central Chile, mainly in December 2022 (and in January 2023; Table 1). We targeted the Chilean Coastal Range between ~34°S to ~40°S (approximately between Valdivia and Santiago, Fig. 1A). This relatively old and low altitude chain of forested highlands runs 100–150 kilometres westwards of, and parallel to, the much younger and higher Andean Mountains. This sampling approach emphasised the areas not covered by the Patagonian Ice Sheet during the Pleistocene (Fig. 1 in Parra-Gómez & Fernández 2022), and is, therefore, more suitable for detecting glaciation-intolerant endogean beetles with low dispersal capacity, the latter hindering recolonization of recently deglaciated areas.

2.2 Sampling protocol

Fieldwork followed the methods described for previous studies conducted on Madagascar and Guatemala

Figure 1. (**A**) map of central Chile between Santiago and Valdivia showing the 15 localities where 50 deep soil samples were taken; (**B**) a pit producing the soil sample CH01 (note the piolet used for digging, a sifter used to sift the soil, and one bag of sifted soil ready to be floated in the barrel with water); (**C**) the floatation process, with the floating fraction scooped by a kitchen sieve and deposited on a fine mesh on the ground; (**D**) a standard floated sample after rinsing in water and before being wrapped in two additional layers of thicker cloth; (**E**) two plastic boxes used for sample transportation and temperature/humidity management, each containing 16 floated samples; (**F**) Sun-driven specimen extraction with floated samples spread on chicken wire and placed on top of aluminium pans (note on the background a funnel suspended from a tree, through which water from all pans was filtered daily).

Table 1. List of 50 Chile deep soil samples and 2,037 endogean adult beetles taken in 15 localities using flotation technique. Abbreviations in locality names are **MN**: Monumento Natural, **P**: Parque, **PN**: Parque Nacional, **RN**: Reserva Nacional, **SN**: Santuario de la Naturaleza. Altitude is given in meters above sea level. The date is either December 2022 (xii) or January 2023 (i). Four-letter subfamily abbreviations for Curculionidae are Coss.: Cossoninae, Cycl.: Cyclominae, and Raym.: Raymondionyminae; those for Staphylinidae are Aleo.: Aleocharinae, Euae.: Euaesthetinae, Lept.: Leptotyphlinae, Osor.: Osoriinae, Psel.: Pselaphinae, Scyd.: Scydmaeninae, and Stap.: Staphylininae. Larvae were detected for Carabidae (one in CH07), Staphylinidae: Leptotyphlinae (one in CH40 and two in CH43) and Osoriinae (one in each CH03 and CH06). All 190 specimens selected for DNA barcoding are coded as light grey (one specimen from a series), dark grey (two specimens) or dark grey with borders (three specimens; Carabidae for CH07 include one larva). All specimens reported in this table are illustrated in Supplementary online material File 3. Note: Printed labels accompanying specimens from samples CH11–CH14 (Llancahue), CH33–CH35 (Santa Juana), and CH36–CH38 (PN Nonguén) contain a typo indicating November (xi), rather than December (xii), as a sampling month; subsequent re-labelling was impossible because specimens have been already distributed among collaborators.

	Locality	Lat.	Lon.	Alt.	Date	Carabidae	Corylophidae	Curculionidae			Elateroidea	Eupsilobiidae	Leiodidae	Ptiliidae	Scarabaeidae	Staphylinidae							Tenebrionidae	Zopheridae	
								Coss.	Cycl.	Raym.						Aleo.	Euae.	Lept.	Osor.	Psel.	Scyd.	Stap.			Σ
CH01	PN Alerce Costero	-39,960	-73,537	25	15.xii													4	1	1					6
CH02	PN Alerce Costero	-39,961	-73,538	22	15.xii													9		1	1	1			12
CH03	PN Alerce Costero	-39,959	-73,532	43	15.xii								39					1	103	4	3				150
CH04	PN Alerce Costero	-39,960	-73,529	48	15.xii													13		3					16
CH05	PN Alerce Costero	-39,951	-73,554	24	16.xii				1				3					25		9		1			39
CH06	PN Alerce Costero	-39,948	-73,554	61	16.xii								3			1		24	19	6					53
CH07	PN Alerce Costero	-39,952	-73,554	20	16.xii	2									4			1	19					4	30
CH08	P Oncol	-39,701	-73,308	514	17.xii				3				22		1		1	23		21	1			1	73
CH09	P Oncol	-39,700	-73,319	533	17.xii	1							10		1		2	23		4					41
CH10	P Oncol	-39,703	-73,311	496	17.xii				2				2												4
CH11	SN Llancahue	-39,857	-73,137	329	18.xii								2							1					3
CH12	SN Llancahue	-39,858	-73,137	330	18.xii								1					20		17					38
CH13	SN Llancahue	-39,854	-73,143	228	19.xii											2	3	28		9		1			43
CH14	SN Llancahue	-39,854	-73,143	232	19.xii	2							11			19		22		1	1				56
CH15	Villa Araucarias	-38,508	-73,216	687	20.xii													64				15			79
CH16	Villa Araucarias	-38,506	-73,218	675	20.xii								5					6		1		2			14
CH17	Villa Araucarias	-38,509	-73,216	694	20.xii															1					1
CH18	Villa Araucarias	-38,520	-73,238	630	21.xii													80		7		3			90
CH19	Villa Araucarias	-38,521	-73,239	630	21.xii												1	16							17
CH20	P Rucamanque	-38,659	-72,601	343	22.xii													17		3					20
CH21	P Rucamanque	-38,659	-72,600	342	22.xii								3					22		10		1			36
CH22	P Rucamanque	-38,666	-72,605	248	23.xii								2					5				2			9
CH23	P Rucamanque	-38,664	-72,602	256	23.xii								3				6	17		21					47

	Locality	Lat.	Lon.	Alt.	Date	Carabidae	Corylophidae	Curculionidae			Elateroidea	Eupsilobiidae	Leiodidae	Ptiliidae	Scarabaeidae	Staphylinidae							Tenebrionidae	Zopheridae	
								Coss.	Cycl.	Raym.						Aleo.	Euae.	Lept.	Osor.	Psel.	Scyd.	Stap.			Σ
CH24	P Rucamanque	-38,665	-72,602	257	23.xii								1					23		3					27
CH25	P Rucamanque	-38,664	-72,602	263	23.xii	3							5	1				19				4			32
CH26	SN El Natri	-37,900	-73,284	89	24.xii	1												8			2				11
CH27	SN El Natri	-37,900	-73,284	98	24.xii								3					53		2	5	1			64
CH28	MN Contulmo	-38,013	-73,185	409	25.xii								16				2	16		1					35
CH29	MN Contulmo	-38,017	-73,177	406	25.xii								1		1		3	32		2					39
CH30	PN Nahuelbuta	-37,828	-73,011	1117	26.xii								1					10		7					18
CH31	PN Nahuelbuta	-37,827	-73,010	1126	26.xii								3					65		16					84
CH32	PN Nahuelbuta	-37,829	-73,009	1118	26.xii								41					54		3					98
CH33	Santa Juana	-37,200	-72,999	154	27.xii								7					4		1					12
CH34	Santa Juana	-37,198	-72,998	169	27.xii								5				1	5		2				1	14
CH35	Santa Juana	-37,199	-72,999	161	27.xii	7							34		72			8		3					124
CH36	PN Nonguén	-36,878	-72,991	84	28.xii	2							9					8		22					41
CH37	PN Nonguén	-36,880	-72,988	103	28.xii	1							35		7			3		3					49
CH38	PN Nonguén	-36,878	-72,994	142	28.xii								58					8		5					71
CH39	RN Los Queules	-35,987	-72,694	472	29.xii													8		3					11
CH40	RN Los Queules	-35,987	-72,695	465	29.xii	1				1			3					27		4					36
CH41	RN Los Ruiles	-35,834	-72,509	213	30.xii					3	2		7					16		106	1			1	136
CH42	RN Los Ruiles	-35,834	-72,508	213	30.xii	2							1					4		24			14		45
CH43	RN Los Ruiles	-35,830	-72,507	367	30.xii	2				3	1		17					75		12	2		3	1	116
CH44	SN Cerro Poqui	-34,207	-71,061	532	31.xii			1										1	5				2		9
CH45	SN Cerro Poqui	-34,206	-71,062	558	31.xii		1											45		3					49
CH46	SN Cerro Poqui	-34,207	-71,061	529	31.xii		1											6		1					8
CH47	SN San Juan de Piche	-33,933	-71,051	620	1.i	2											7	2	3					2	16
CH48	SN San Juan de Piche	-33,934	-71,051	592	1.i																			3	3
CH49	Curico	-35,069	-71,113	475	3.i																		3	1	4
CH50	Curico	-35,067	-71,118	378	3.i							1		2					1				3	1	8
					Σ	26	2	1	6	7	3	1	353	3	86	22	26	920	151	343	16	31	25	15	

(Andújar & Grebennikov 2021, Barrios-Izás et al. 2024). We sampled 15 Chilean localities (Fig. 1A), each of them covered by natural forest. Any two nearest sampled localities were separated by 15–135 kilometres. Each of the 15 localities produced between two and seven soil samples taken minimally 20 and maximally 1,500 meters apart; 50 samples were taken in total (Table 1). The soil was dug by a piolet (Fig. 1B), i.e., a two-headed ice axe used in mountaineering. Prior to soil sampling, the litter and the top 5–10 cm of the notably darker organic-rich upper soil were removed from the area of some four square meters, to expose deeper and lighter mineral soil which is notably poorer in organic content. A pit was dug (Fig. 1B) some 40 to 100 cm deep, to produce the deep soil sample (Supplementary online material File 1). This soil was sifted on the spot through a mesh of one cm square (Fig. 1B) to remove stones and larger organic particles such as roots and twigs. Three to five bags of sifted soil, each approximately 30 kg in mass (Fig. 1B), were transported to the nearby source of freshwater and floated in a barrel some 100 litres in volume (Fig. 1C). Soil was sunk in the water and energetically stirred a few times, to allow its organic content (including endogean beetles) to float. This floating substance formed a foam which was scooped by a kitchen sieve some 20 centimetres in diameter and placed in the middle of a rectangular fine mesh (cut from window curtains) some 1–2 square meters in size (Fig. 1C). This mesh and its content were rinsed in fresh running water for at least a minute, to remove the smallest soil particles, as judged by the clarity of the water filtering through the sample (Fig. 1D). Water was removed from the sample, first by letting it filter through and then by rotating the sample wrapped in the mesh in a vertical plane some 5–10 times to utilize the centrifugal force. So treated, the sample had an appearance of a ball of some 20–25 centimetres in diameter and some 2–4 kilograms in weight. The wet sample in the mesh was wrapped in two pieces of cloth (cut from linens) comparable in size with the mesh, to form a slightly wet protective cocoon around the sample. Care was taken to document samples by showing pre-printed sample numbers on sampling site photographs and keeping these numbers together with the sample all the time, including the extraction period (see below). So secured, floated samples were stored and transported for up to two weeks in closed plastic boxes in the trunk of a car, each box holding 16 samples arranged in two layers (Fig. 1E). Every 2–3 days, samples were sprinkled with water and gently massaged, to maintain moisture and the airflow inside them. Constant care was taken not to overheat the samples, particularly when parking the car with the samples during the day. The air temperature around the samples was monitored (Fig. 1E) and varied between +9˚C early in the mornings (after samples being exposed the whole night to the cool night air) and +18˚C in the afternoons (after spending most of the day in boxes inside the car; boxed additionally wrapped in blankets). A total of 18 days passed between the first sampling day (December 15) and the day when the samples were first exposed to the Sun (January 2). Due to the care taken with the samples, we assumed that no significant dying out of organisms took place during this period.

2.3 Specimen extraction protocol

For extraction of live endogean beetles, the driest, hottest and sunniest nearby locality was selected (near Curicó: 35.0623°S 71.1657°W). On January 2, we individually spread 48 floated samples (two more were sampled and added the next day) into a layer of some 2–4 cm in thickness over the chicken wire rectangles (1⁄4 or 1/2 inch mesh size; Fig. 1F). Fine cloth mesh with holes about 1–3 mm in linear size was used to support the samples on chicken wire. These chicken wire rectangles with floated samples on them were then placed on top of 50 aluminium pans measuring 53.34 x 33.02 x 7.62 cm (21 x 13 x 3 inches) and each containing 1.5 litres of water. For the next six days, the samples were exposed to the sun to gradually dry from above. This gradual process was expected to drive the heat- and dryness-intolerant soil organisms downwards, through the chicken wire and into the water below. Each day water from 50 aluminium pans was filtered through a funnel with the fine mesh to extract and preserve live and recently drowned soil organisms in 96% ethanol. This procedure was conducted in the afternoon as close to the sunset as possible (i.e., not in the morning). This timing minimises the growth of fungi on dead specimens (which would be much more significant, if this daily cycle starts in the morning, followed by a hot day when specimen extraction is likely most active). The average high air temperature during the extraction period was 29˚–30˚C, mostly sunny, with no rain. The maximal temperature of the upper surface of the soil samples exposed to the Sun exceeded 50˚C. During the first 4–5 days about half of 50 soil samples were completely dry and discarded; during the last (sixth; January 8) extraction day only ten most voluminous soil samples retained some moisture and were still being processed.

2.4 Specimen sorting

We used a dissecting microscope to separate endogean beetles from non-endogean ones. The latter formed a bycatch of our sampling protocol formed by specimens falling from the soil surface, leaf litter, and perhaps from the canopy. Separation of endogean beetles was based on three easily observable external morphological characteristics: (1.) small or absent eyes; (2.) relatively small body, and (3.) uniformly pale body colour. Edaphic and litter-dwelling Pselaphinae and Scydmaeninae having small and pale bodies were difficult to categorize as endogean or litter-inhabiting. For them, specimens with the smallest eyes were selected as endogean and those with larger eyes were not; this distinction is partly arbitrary and, therefore, is difficult to formalize and replicate.

2.5 Sampling statistics

Endogean adult beetles (and six larvae presumably belonging to them) were sorted into 19 broad taxonomic units consisting of 11 families and, for species-rich Curculionidae and Staphylinidae, of 10 subfamilies (19 taxonomic units in total: either a family or a subfamily). We tabulated the number of adult beetles in each of the 50 soil samples for each of the 19 taxonomic units (Table 1) and illustrated each cell in the Table 1 containing specimen records (Supplementary online material File 2). To compare the diversity of endogean beetles between samples and between localities, we calculated the average number of taxonomic units detected in each sample and in each locality (irrespective of the absolute number of specimens). In addition, we assumed each of 50 soil samples to weigh 120 kg (no exact measurements were taken) and calculated the average number of specimens collected per kilogram of deep soil per locality and overall.

2.6 Specimen identification

Like with any organism, taxonomic identification of Chilean endogean beetles is possible only as far as their names exist and can be readily used (i.e., without undertaking labour-intense taxonomic revisions; the latter are outside of the scope of this sampling report). Some of our specimens are obviously unnamed (=new to science) and, therefore, unidentifiable beyond a genus, tribe, subfamily, and, in one notable instance, beyond the superfamily (Elateroidea, Fig. 2) level. Others belong to groups where at least generic names are likely available, but their use is hampered by the lack of adequate taxonomic information and the likelihood of encountering an unnamed taxon (e.g., Staphylinidae: Leptotyphlinae). To achieve the best possible taxonomic identification for our unknown or inadequately known Chilean endogean beetles, we solicited the opinions of entomological colleagues by sending them specimen pictures (see Acknowledgement). We critically evaluated the opinions of our colleagues, and either accepted or modified/rejected them. Existing taxonomic names can often be used with a variable degree of accuracy and, therefore, taxonomic experts were sometimes reluctant to express a definite opinion (e.g., in Staphylinidae: Pselaphinae). In such cases, we often preferred to use these generic names with our specimens and highlight the preliminary nature of such identifications. All correct identification, therefore, should be attributed to the taxonomic experts listed in the Acknowledgement, while all possible misidentifications are ours. We refer to raymondionymine weevils as the subfamily Raymondionyminae, following Andújar et al. (2024), and not as a tribe of the subfamily Brachycerinae.

2.7 DNA barcoding and analysis

To bypass the peril of inadequate taxonomy of Chilean endogean beetles and facilitate their future studies, we followed other authors working with inadequately known arthropod faunas (e.g., beetle myrmecophiles of Costa Rica by von Beeren et al. 2023, or Appalachian leaf litter arthropods by Caterino & Recuero 2024, or Taiwan leaf litter beetles by Hu et al. 2024) and provided a DNA barcode library. One hundred ninety specimens of Chilean endogean beetles (all adults, except for one carabid larva from CH07) were selected for DNA barcoding (Supplementary online material File 3). We sequenced them for up to 658 bp of the 5' end of the mitochondrial cytochrome c oxidase I gene (Hebert et al. 2003). Non-destructive DNA extractions were performed after disarticulate each voucher using MAg-bind Blood & Tissue DNA extraction Kit (Omega Bio-tek) in a KingFisher robotic system (Thermo Fisher Scientific inc.) following the manufacturer protocol. PCR amplification was done for the 5' end COI gene (standard barcode region for Metazoa; Hebert et al. (2003)) using degenerate Folmer barcode primers (Fol-degen-for: 'TCNACNAAYCAYAARRAYATYGG'; Fol-degen-rev: 'TANACYTCNGGRTGNCCRAARAAYCA'; Folmer et al. (1994), Yu et al. (2012)). PCR reactions were done using from 1ul to 5µl of extracted DNA and 18 µl of PCR mix, which consisted in: 12.72 µl molecular-grade water, 2 µl10x NH buffer, 1.2 µl MgCl , 0.4 µl dNTPs, 0.4 µl of BSA, 0.6 µl 10 µM of each primer, and 0.08 µl Taq polymerase (BIOTAQ™ DNA Polymerase, Bioline) per sample. PCR conditions were: 10 min at 95°C in 10 min, followed by 40 cycles of 30 s at 95°C, 30 s at 48°C and 3 min at 72°C; 10 min at 72°C and holding at 10°C. PCR products were inspected in agarose gel 1%. For those

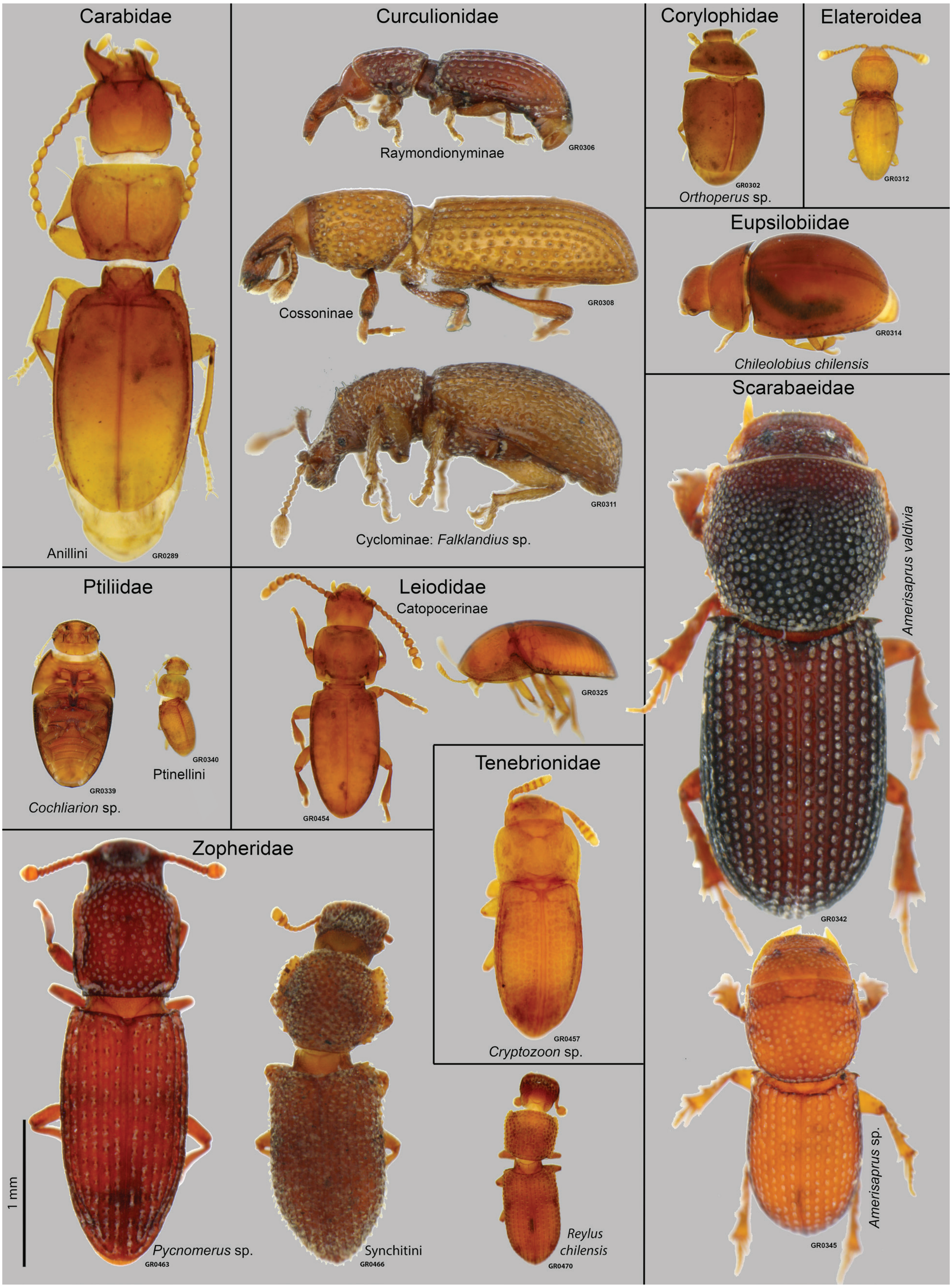


Figure 2. Endogean non-Staphylinidae beetles of Chile.

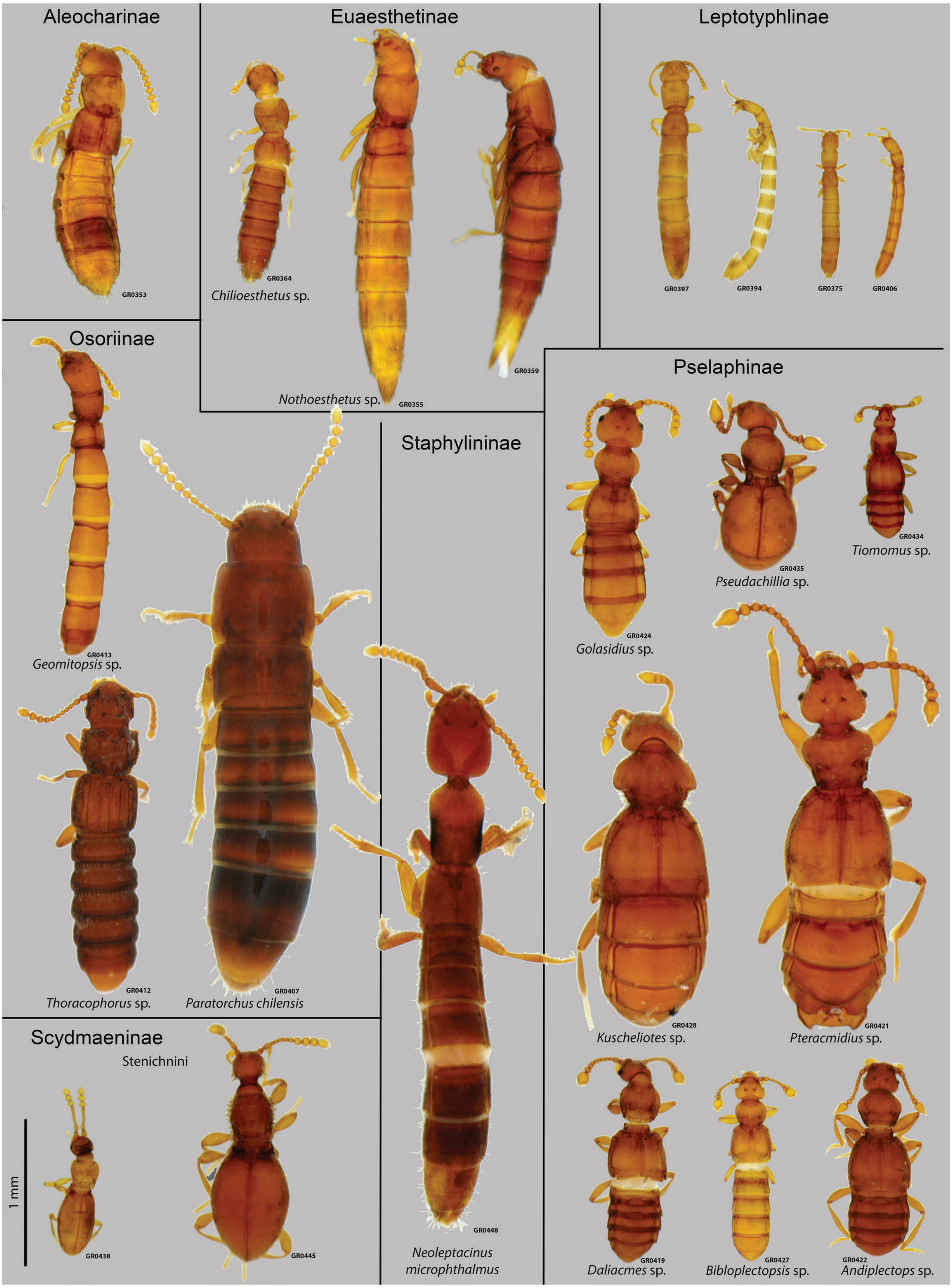


Figure 3. Endogean rove beetles (Staphylinidae) of Chile.

samples failing to amplify, we conducted new PCR for a second fragment of 418bp using the same reverse primer (Fol-degen-rev) combined with forward primer Ill_B_F: 'CCIGAYATRGCITTYCCICG' (Shokralla et al. 2015). PCR reactions were conducted as before. Amplicons were sent for purification and Sanger-sequencing by Macrogen, Spain. Three among 190 specimens failed (GR0323: Leiodidae: Catopocerinae, GR0346 Scarabaeidae: Aphodiinae: *Amerisaprus*, and GR0428 Staphylinidae: Pselaphinae: *Kuscheliotes*); the remaining 187 gave sequences longer than 200 base pairs (183 of them longer than 400 base pairs; the GenBank accession numbers are PP351462–PP351648. Information about these 190 specimens (including locality data, specimen image, and DNA barcode sequence) is available online in a public dataset dx.doi.org/10.5883/DS-VGDS28 in the Barcode of Life database (=BOLD, Ratnasingham & Hebert 2007, http://www.boldsystems.org/).

We used the Barcode Index Numbers (BINs, Ratnasingham & Hebert 2013) for grouping DNA-barcoded specimens into operational taxonomic units approximately corresponding to species. We performed a simplified phylogenetic analysis of 187 newly sequenced DNA barcodes with the main purpose to visualize taxonomic or geographic grouping of Chilean endogean beetles. Because Staphylinidae DNA barcode sequences accounted for approximately half of those we generated, we divided our data into two identical analyses with the practical purpose of fitting each topology in one page: 102 Staphylinidae and 85 non-Staphylinidae endogean Chilean beetles, respectively. Both topologies were built with the maximum likelihood (ML) approach, as implemented in CIPRES Science Gateway online platform (Miller et al. 2010; http://www.phylo.org/, tool 'RAxML-HPC BlackBox (8.2.12) - Phylogenetic tree inference using maximum likelihood/rapid bootstrapping on XSEDE.', Stamatakis 2014) and default values. Both trees were visualised in FigTree v1.4.4 (Rambaut 2020). We rooted the non-Staphylinidae topology between the phylogenetically most distant Adephaga (Carabidae) and Polyphaga; the Staphylinidae topology was rooted between monophyletic Leptotyphlinae and the rest of the family.

2.8 Specimen illustration

We provide habitus photographs of all reported beetles found in our Chilean deep soil samples (Figs 2–5, Supplementary online material Files 2 and 3). Specimen images were taken with Nicon DXM1200F digital camera attached to a dissecting scope (Figs 2–3). Uncoated specimens for SEM were glued to points of #3 entomological pins, placed horizontally on a carbon adhesive tab forming the top of a round aluminium stub, and imaged using a Hitachi SU7000 FE-SEM (Hitachi, Tokyo, Japan), and a UVF detector operating at 20 kV, 100 Pa (Figs 4–5).

2.9 Specimen deposition

All specimens reported in this work are presently stored in the Canadian National Collection of Insects, Arachnids and Nematodes, Ottawa, Canada. Selected specimens might be later deposited in other museums, depending on the specific studies resulting from this initial sampling report. We intend to deposit holotypes designated in the future from the herein reported specimens, if any, in the Museo Nacional de Historia Natural, Santiago, Chile.

3. Results

The first 48 soil samples were taken daily between December 18 and January 1, 2024, both days inclusive, and varied between two and four samples per day, with 2.7 being the daily average; samples CH49 and CH50 were added on January 3, 2023. In total, we extracted 2,037 adult endogean beetles and six larvae belonging to 19 family/subfamily taxonomic units (Table 1). The most numerically abundant groups were Leptotyphlinae, Leiodidae and Pselaphinae representing 45 %, 17 %, and 17 % of all specimens, respectively; all remaining beetles constituted 21 %. The number of adult endogean beetles per sample varied between one and 150 (Table 1), with 41 being the average. The diversity of endogean beetles per sample measured in taxonomic units varied between one (samples CH17 and CH48) and nine (sample CH43), with four being the average. The diversity per sample per locality measured in taxonomic units varied between 2.4 (Villa Araucarias) and 7.33 (RN Los Ruiles), with four being the average. The taxonomic diversity per locality measured in taxonomic units varied between three (PN Nahuelbuta) and 11 (PN Alerce Costero), with 6.53 being the average. The average number of specimens collected by sampled kilogram of deep soil was 0.34. Phylogenetic analysis of DNA barcodes resulted in taxonomically and geographically congruent trees (Figs 6, 7).

Carabidae were represented by the trechine tribe Anillini (Figs 2, 4). In total, 26 adults (and one larva GR0285 in sample CH07) were found in 12 samples taken in 10 localities (Table 1). Anillini of South America are poorly known and consist of ten named species attributed to seven genera all endemic to the continent: *Anillotarsus* *tetramerus* Mateu, 1980; *Cryptocharidius mandibularis* Etonti & Mateu, 1992; *Nothanillus germaini* Jeannel, 1962; *N. lui*sae Bonniard de Saludo, 1970; *Paranillopsis pampensis* Cicchino & Roig-Junent, 2001; *P. piguensis* Cicchino & Roig-Junent, 2001; *Perucharidius andinus* Mateu & Etonti, 2002; *P. etontii* Magrini & Benelli, 2018; *Stylulus* (*Stylulites*) *plaumanni* (Jeannel, 1963) and *Zoianillus acutipennis* Sciaky, 1994 (Cicchino & Roig-Juñent 2001, Mateu & Etonti 2002, Magrini & Benelli 2018). The documented anilline diversity of Chile consists of two named species of the genus *Nothanillus* Jeannel, 1962, both eyeless and endemic to the country (Jeannel 1962b, Bonniard de Saludo 1969, Roig-Juñent & Domínguez 2001). Some of our specimens have the 'recurrent striae' on the elytra characteristic to this genus (Supplementary online material File 3) and, therefore, likely belong to it.

Corylophidae were represented by two seemingly conspecific specimens (Fig. 2) from two samples taken in SN Cerro Poqui and likely belonging to the genus *Orthoperus* Stephens, 1829 (Table 1). Because members of the family are 'typically encountered in habitats where moulds and other fungi are common (e.g. under bark, in decaying plant matter, etc.)' and have never been recorded from deep soil (Robertson et al. 2012), our finds might represent an accidental bycatch from the surface (or, they might not, because many beetles without obvious endogean adaptations use deep soil habitats).

Curculionidae: Cossoninae were represented by a single specimen (Fig. 2) found in sample CH44 and belonging to an unidentified genus (Table 1). While Cossoninae are known to contain eyeless deep soil dwellers elsewhere (e.g., West Palaearctic genus *Barretonus* Roudier, 1958, see García et al. 2019), no eyeless members of this subfamily have been previously recorded from Chile.

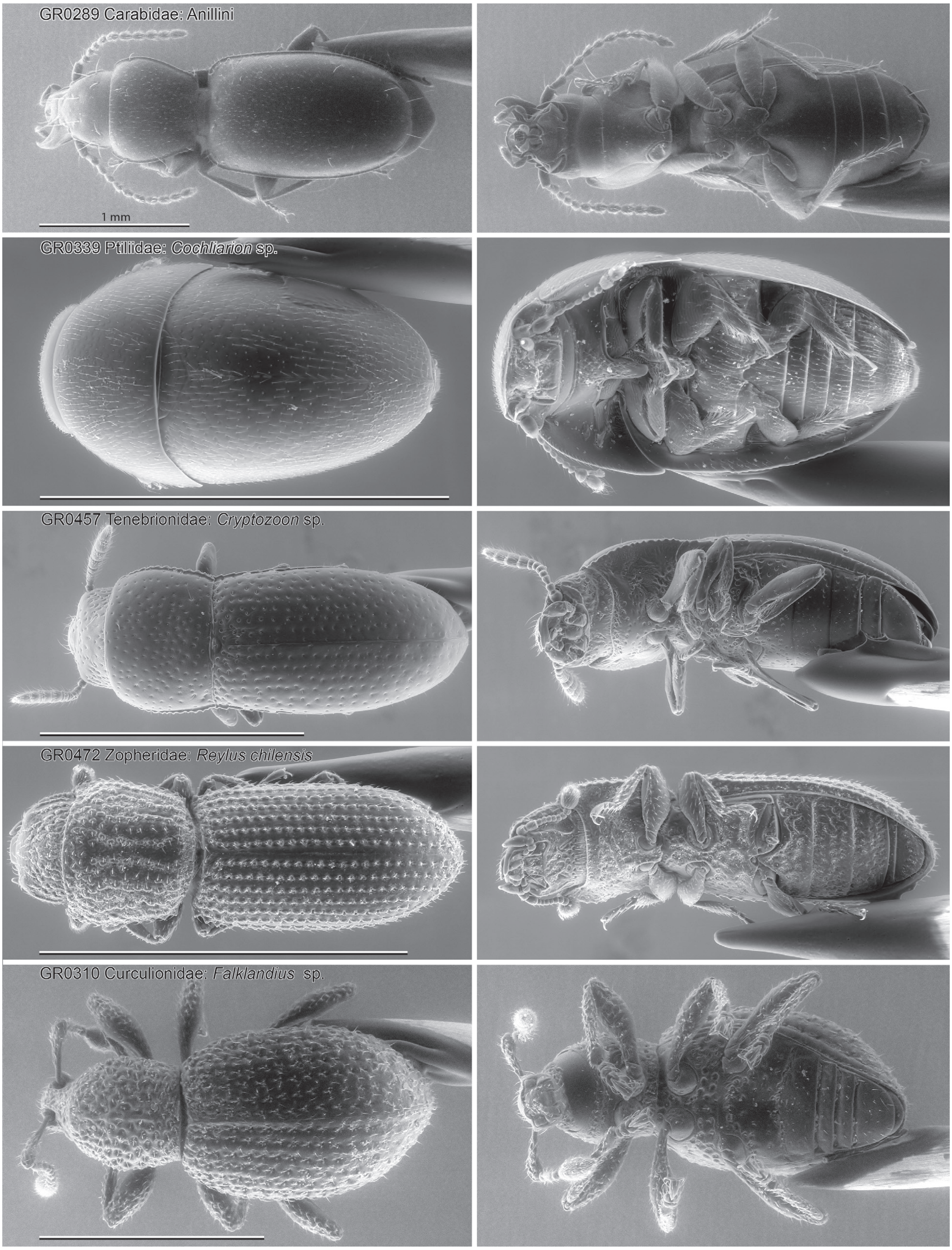
Curculionidae: Cyclominae were represented by six specimens of the listroderine genus *Falklandius* Enderlein, 1907 (Figs 2, 4) found in three samples taken in two localities around Valdivia (Table 1); some of them likely belonging to *F. chilensis* Morrone & Anderson, 1995 (Morrone & Anderson 1995, Morrone 2013).

Curculionidae: Raymondionyminae (*sensu* Andújar et al. 2024) were represented by seven conspecific specimens (Fig. 2) found in three samples taken in two nearby localities (Table 1). The only named eyeless South American member of this subfamily is the genus *Bordoniola* Osella, 1987 with seven species in Venezuela and Ecuador all known only from the type series (Baviera et al. 2012).

Elateroidea were represented by three conspecific specimens (Fig. 2) found in two samples taken in RN Los Ruiles (Table 1). These eyeless beetles have a body length below 1 mm, well-developed promesosternal clicking mechanism, and clubbed antennae. Our attempts to identify a family for these beetles remain inconclusive (Muona et al. 2020; see also Acknowledgement). Presently a DNA-based attempt in underway, to elucidate the phylogenetic identity of these organisms. Suggestively, although the validity of at least some historical families of Elateroidea is doubted (Douglas et al. 2021), two new species-poor families were recently established within Elateroidea for newly discovered organisms, Iberobaeniidae from Spain (Bocak et al. 2016) and Jurasaidae from Brazil (Rosa et al. 2021).

Eupsilobiidae were represented by a single specimen of likely *Chileolobius chilensis* Pakaluk & Ślipiński, 1990 (Fig. 2) from sample CH50. Like those of above-mentioned Corylophidae, members of the family Eupsilobiidae have never been recorded from deep soil (Pakaluk & Ślipiński 1990) and, therefore, our find might represent an accidental by-catch from the surface (or, the specimen collected was indeed sampled from deep soil and this is another novel result).

Leiodidae were represented by 351 eyeless specimens recorded in all but three of the northernmost sampled localities (Table 1). All but three of these specimens (including sequenced specimens GR0315–GR0338, Fig. 6 and Supplementary online material File 3) belong to more than one unnamed species (Alfred Newton, personal communication) of an unnamed genus with peculiarly rounder bodies (Fig. 2 shows this beetle in lateral view). This genus was referred to in the literature for almost four decades (Newton 1985, 1998, 2000; Nishikawa 2018; Perreaut 2019). It was informally called 'the turtle Leiodid' by its discoverer (Newton 2000), is characterized by the peculiarly rounder turtle-like appearance and was thought to be related to the subfamily Catopocerinae (references above). Three other markedly different, slender, and parallel-sided specimens (specimens GR0454 & GR0455 from sample CH22 and specimen GR0456 from sample CH41, Figs 2 and 6), all sympatric with the turtle genus, seem to belong to yet another and previously unknown genus, also with catopocerine affinities (Alfred Newton, personal communication). Named members of this subfamily are known only from the United States (three genera, Peck & Cook 2011) and Eastern Palaearctic (three more genera, including one extinct, Perreau & Růžička 2007, Nishikawa 2018, Perreau 2019), which suggests an interesting biogeographic puzzle.



GR0289 Carabidae: Anillini

GR0339 Ptiliidae: *Cochliarion* sp.

GR0457 Tenebrionidae: *Cryptozoon* sp.

GR0472 Zopheridae: *Reylus chilensis*

GR0310 Curculionidae: *Falklandius* sp.

Figure 4. SEM images of endogean non-Staphylinidae beetles of Chile.

Ptiliidae were represented by three specimens found in two samples taken in two localities (Table 1). The relatively large and rounded specimen in sample CH25 likely belongs to the genus *Cochliarion* Deane, 1930 (Figs 2, 4) with a single named species in Australia. The genus has been reported previously from Chile as potentially adventive based on a single record in a *Eucalyptus* forest (Newton 1985). Subsequent Chilean records (Alfred Newton, personal communication) and our record from natural environments indicate a wider distribution and the possibility of its native presence in Chile. Two conspecific specimens in sample CH50 likely belong to an unidentified genus of Ptiliinae: Ptinellini (Fig. 2).

Scarabaeidae are represented by 86 specimens found in six samples taken in five localities (Table 1). They all belong to the aphodiine genus *Amerisaprus* Stebnicka and Skelley, 2003. Its only named species, *A. valdivia* Stebnicka and Skelley, 2003, is known only from the type series. The type locality is some 35 kilometres from our sample CH07 containing four specimens of likely the same species (Fig. 2; plus two additional likely conspecific specimens in samples CH08 and CH09). A smaller and paler unnamed species likely belonging to the same genus was sampled further north (Fig. 2; samples CH29 and CH35); both these *Amerisaprus* species were detected in sample CH37. Our attempt to

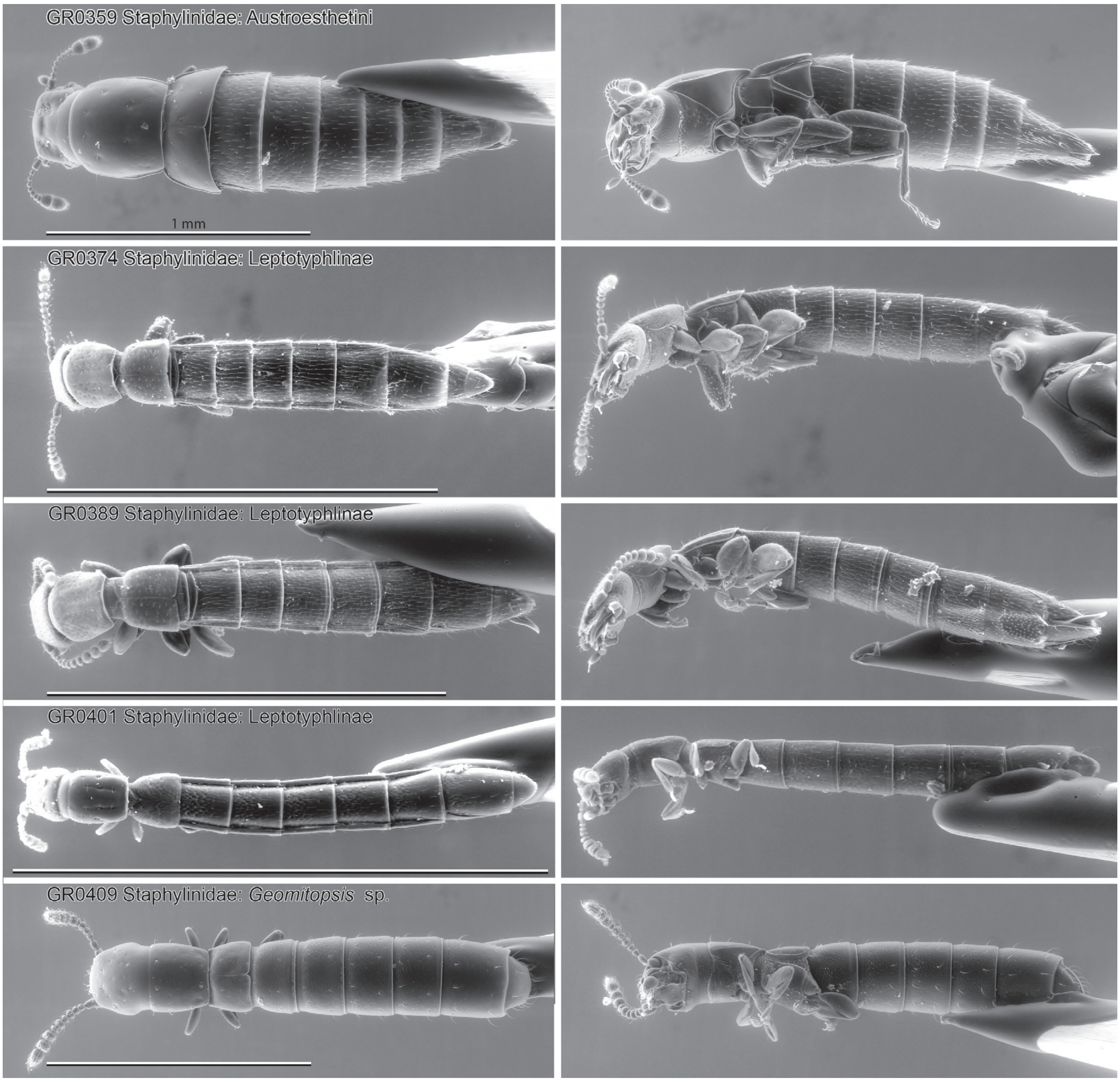


Figure 5. SEM images of endogean rove beetles (Staphylinidae) of Chile.

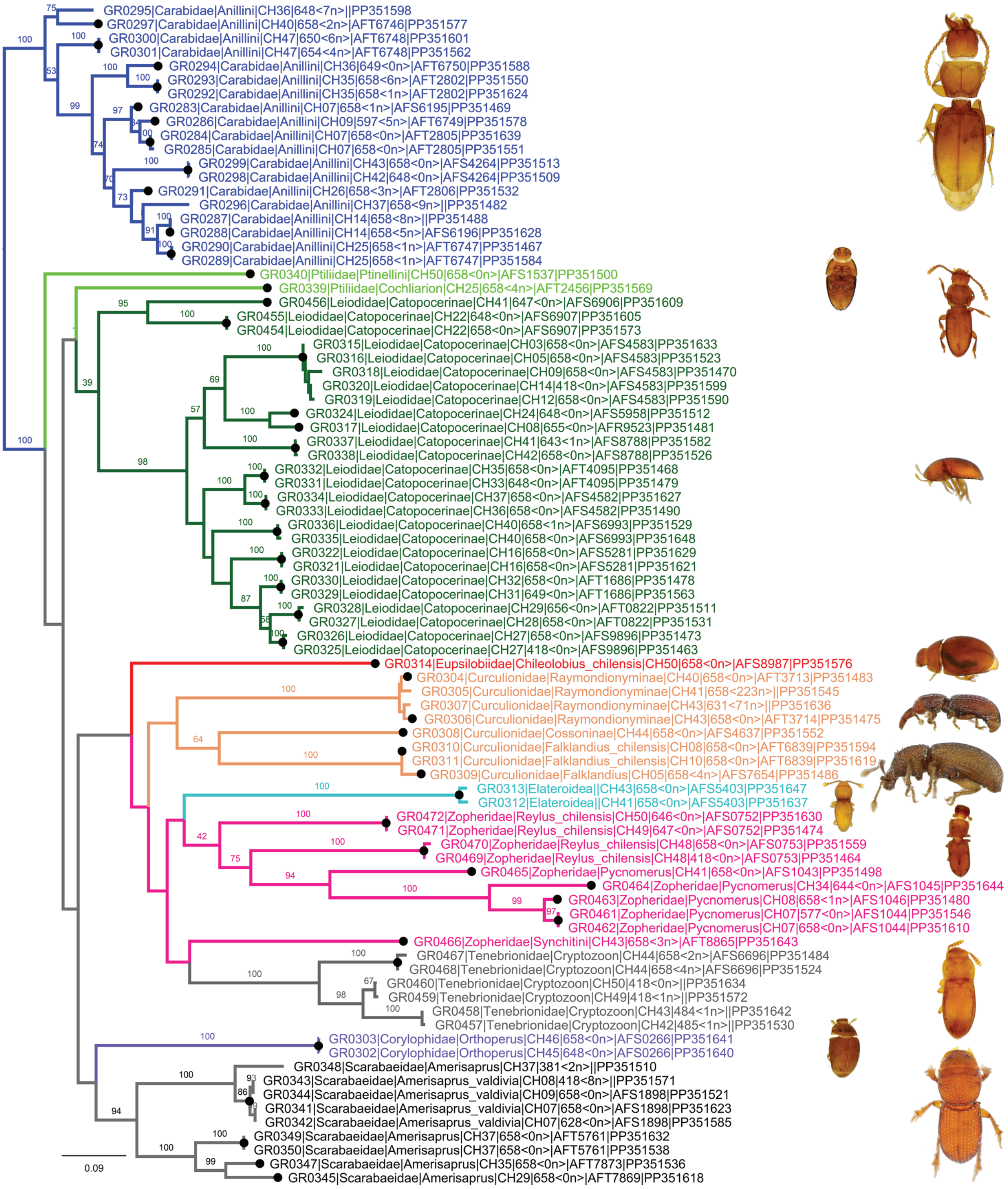


Figure 6. Maximum Likelihood DNA barcode tree of 85 non-Staphylinidae endogean beetles of Chile. Families are colour coded. Terminal names consist of specimen number, the family name (superfamily for specimens GR0312 and GR0313), the most detailed current taxonomic identification (species, genus, tribe, or subfamily), sample number, length of the DNA barcode fragment [with the number of ambiguously read bases in angle brackets], BIN number [if applicable, also denoted on the tree with black dots], and GenBank accession number. Digits at internodes are rapid bootstrap values of 50 % and above.

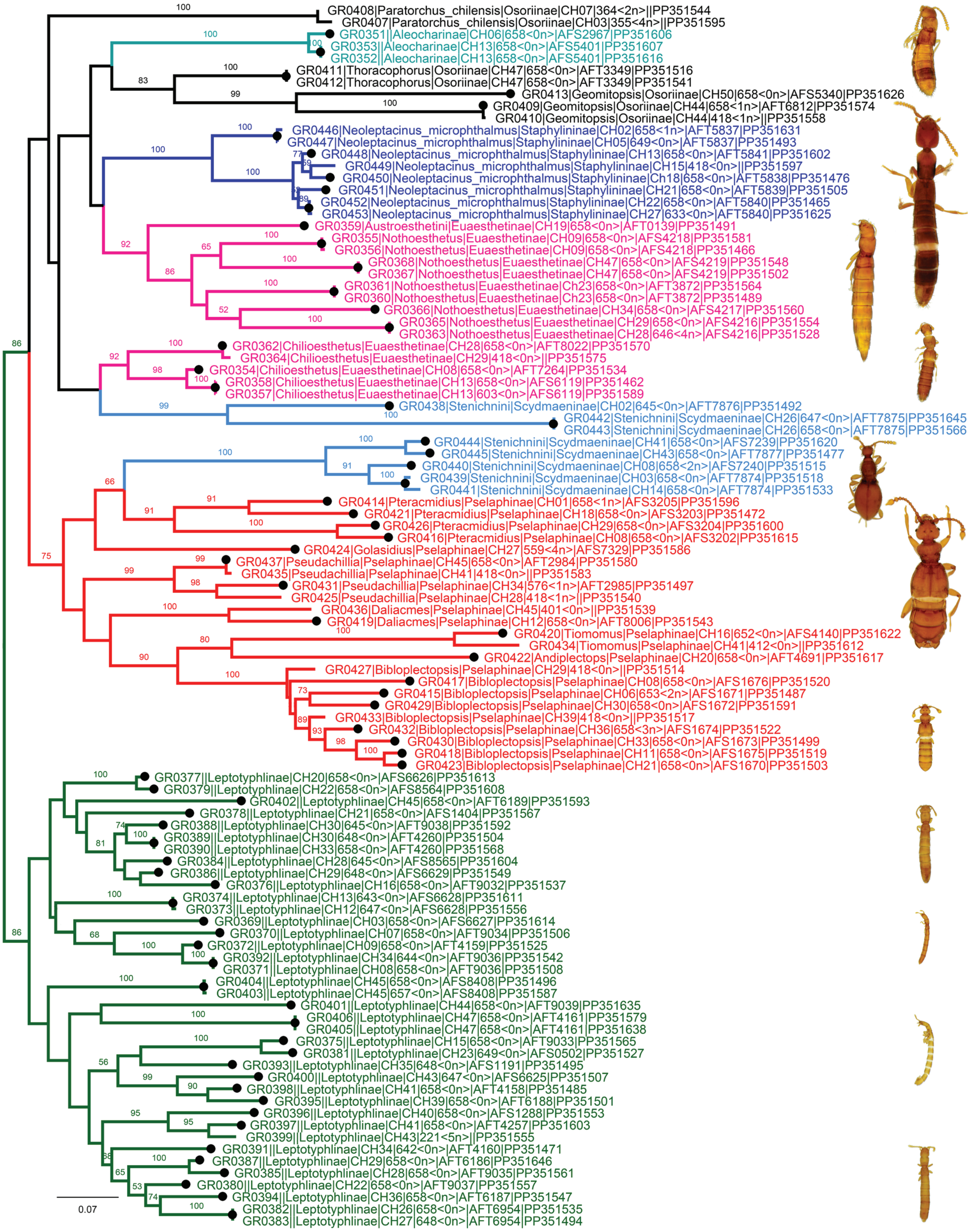


Figure 7. Maximum Likelihood DNA barcode tree of 102 endogean rove beetles (Staphylinidae) of Chile. Subfamilies are colour coded. Terminal names consist of the specimen number, the most detailed current taxonomic identification (species, genus, tribe, or subfamily), sample number, length of the DNA barcode fragment [with the number of ambiguously read bases in angle brackets], BIN number [if applicable, also denoted on the tree with black dots], and GenBank accession number. Digits at internodes are rapid bootstrap values of 50% and above.

find a larva of this genus, particularly in sample CH35 containing a whopping 72 adults of the smaller species, was unsuccessful.

Staphylinidae: Aleocharinae are represented by 22 seemingly conspecific specimens found in three samples taken in two localities adjacent to Valdivia (Fig. 3; Table 1); their more precise identification requires a dedicated study and, therefore, is not attempted.

Staphylinidae: Euaesthetinae were resented by 26 specimens found in nine samples taken in seven localities (Table 1); all but one of them belong to the genera *Chilioesthetus* Sáiz, 1968 (Fig. 3; CH08, CH13) and *Nothoesthetus* Sáiz, 1970 (Fig. 3; CH09, CH34, CH47). The specimen from sample CH19 (Figs 3, 5) likely represents an unnamed genus already known from Chile (David Clarke, personal communication). Interestingly, samples CH28 and CH29 each contain specimens of both named genera.

Staphylinidae: Leptotyphlinae were represented by 920 specimens found in 44 samples taken in all but one sampled locality (Figs 3, 5; Table 1). These beetles account for 45 % of all herein reported deep soil specimens. There larvae were sampled together with adults, in samples CH40 and CH43. Sáiz (1974) published a key to all six Chilean Leptotyphlinae genera, all endemic to the country. Except a larval description (Grebennikov & Newton 2009), no subsequent research on the Chilean members of the subfamily has been published.

Staphylinidae: Osoriinae were represented by 151 specimens found in seven samples taken in four localities (Table 1) and belonging to three genera and three species. All 142 relatively large microphthalmic specimens (Fig. 3) from PN Alerce Costero are either conspecific with (and in this case represent the northernmost records of), or most closely related to, *Paratorchus chilensis* (Irmler, 2005). Our two records of a small and eyeless species (CH44 and CH50) likely belong to the genus *Geomitopsis* Scheerpeltz, 1931 (Figs 3, 5). This is suggested by the peculiar pattern of alternating large and small antennomeres detectable on our specimens and resembling those of that genus (Alfred Newton, personal communication), with at least two species known from Chile: *G. campanae* Saiz, 1973 and *G. chilensis* Coiffait and Saiz, 1965 (fig. 2 in Sáiz 1973). Some 20 species of the genus *Geomitopsis* are scattered in southern Europe, Africa, and South America (Herman 2001a, Irmler 2016), which is a peculiarly wide distribution for supposedly low-dispersal eyeless and wingless soil-adapted organisms (Pérez-Delgado et al. 2022). Finally, all three conspecific specimens in sample CH47 belong to the nearly cosmopolitan genus *Thoracophorus* Motschulsky, 1837. Our specimens (Fig. 3) have evenly rounded anterior head margins when viewed from above, distinguishing them from Chilean *T. araucoensis* Irmler, 2001 (fig. 9a in Irmler 2001). The only other named Chilean congener, *T. bonvouloiri* (Fauvel, 1867) described from Santiago, is too inadequately known to be compared with our specimens.

Staphylinidae: Pselaphinae were represented by 343 specimens found in 38 samples taken in all but two localities. Excepting the genera *Pseudachillia* Jeannel, 1963 (Fig. 3) and *Golasidius* Jeannel, 1962 (Fig. 3), belonging to the supertribes Goniaceritae (Brachyglutini) and Faronitae, respectively, the rest of these specimens belongs to the supertibe Euplectinae. The tribe Jubini is represented by the genus *Kuscheliotes* Jeannel, 1962 (Fig. 3), while the tribe Trichonychini is represented by five genera: *Andiplectops* Jeannel, 1962 (Fig. 3), *Bibloplectopsis* Jeannel, 1962 (Fig. 3), *Daliacmes* Jeannel, 1962 (Fig. 3), *Pteracmidius* Jeannel, 1962 (Fig. 3), and *Tiomomus* Jeannel, 1962 (Fig. 3). Jeannel's (1962a) treatment of these beetles remains the main identification source, while an updated list of Chilean genera and species was provided by Asenjo et al. (2019).

Staphylinidae: Scydmaeninae were represented by 16 specimens found in eight samples taken in five localities (Table 1). All of them belong to the tribe Stenichnini (Fig. 3), most likely to the genera *Euconnus* Thomson, 1859, *Magellanoconnus* Jałoszyński & Newton 2017, and *Sciacharis* Broun, 1893 (Jałoszyński & Newton 2017).

Staphylinidae: Staphylininae were represented by 31 specimens found in ten samples taken in five localities (Table 1) and all likely belonging to the microphthalmic xantholinine species *Neoleptacinus microphthalmus* Saíz, 1968 (Fig. 3).

Tenebrionidae were represented by 25 specimens found in six samples taken in three northernmost localities (Table 1). All of them likely belong to the genus *Cryptozoon* Schaufuss, 1882 (Figs 2, 4; Diaperinae: Gnathidiini: Anopidiina) (Spiessberger & Ivie 2020). The genus and the tribe were not previously recorded from Chile (Vidal & Guerrero 2007) and, therefore, our specimens likely belong to at least one unnamed species.

Zopheridae were represented by 15 specimens found in nine samples taken in six localities (Table 1). Those from samples CH07, CH08, CH34, and CH41 belong to the nearly cosmopolitan genus *Pycnomerus* Erichson, 1842 (Fig. 2; Zopherinae). The single specimen CH43 is an unknown member of the tribe Synchitini in need of further research (Fig. 2). Those from samples CH47–CH50 are either conspecific, or congeneric, with *Reylus chilensis* (Dajoz, 1969), the sole species of its genus (Colydiinae; Figs 2, 4 and fig. 49 in Ivie et al. 2016).

Eyeless Chilean beetles not detected by us. We did not find two species of eyeless beetles described from Chile and known only from the type series: *Amydropa anophthalma* Reitter, 1877 (Cryptophagidae, Leschen 1996) and *Neotorneuma porteri* Hustache 1939 (Curculionidae, Howden 1992, Elgueta & Marvaldi 2006).

4. Discussion

The main hypothesis tested by our deep soil sampling in Chile was whether this specialized sampling methodology can detect a significant number of endogean beetles, with at least some of them representing lineages new to science. This hypothesis seems to be fully vindicated by the following list of what presently appear to be the most significant novelties. Firstly, three Elateroidea specimens from samples CH41 and CH43 cannot at present be attributed to any family using their morphological characters. Secondly, our records of raymondionymine weevils in samples CH40, CH41 and CH43 are first for the country and more than 3,500 km further south from the nearest known localities for this subfamily (although Canadian Museum of Nature [CMN] collection contains a similar specimen collected by Tomás Cekalovic on December 22, 1994, in Cerro Caracol, Concepcion, Chile). Thirdly, two unnamed genera of eyeless Leiodidae, one of them previously known and another entirely unexpected, represent phylogenetically unsettled taxonomic and biogeographic novelties. Fourthly, the darkling beetle genus *Cryptozoon* newly reported from Chile eluded earlier thorough surveys of the family (Vidal & Guerrero 2007). Fifthly, the re-discovery of the aphodiine genus *Amerisaprus* and the discovery of what looks like its second and even more morphologically soil-adapted species, are novel. Finally, our numerically rich and genetically diversified samples of Anillini (forming 11 BINs at BOLD Systems) and Leptotyphlinae (31 BINs) greatly exceed all previous reports for these organisms in Chile. In short, and apart from the likely non-endogean Corylophidae and Eupsilobiidae, any of the remaining 17 higher taxonomic units (Table 1) contain significant taxonomic, faunal, biogeographic, and phylogenetic novelties. We conclude, therefore, that (1.) the Coastal Range in Chile harbours a diverse fauna of endogean beetles comparable in species number with those of the better-studied Mediterranean region, while (2.) our relatively massive in volume and partially novel in design deep soil beetle sampling procedure is highly productive.

Our study suggests new intellectually stimulating directions for further research. Firstly, what is the full extent of endogean beetle diversity in Chile? Secondly, what are the phylogenetic affinities, biogeographic history, and taxonomic placement of these supposedly low-dispersing organisms? Thirdly, what is the footprint of the Last Glacial Maximum and the subsequent retreat of the Patagonian Ice Sheet on the distribution of endogean beetles in the south of Chile? Lastly, how far north can endogean beetles be found in Chile, considering the climatic extremes of the northernmost third of the country occupied by the hyper-arid and ancient (Hartley et al. 2005) Atacama Desert? This inhospitable region has a meridional chain of highly fragmented fog oases (Moat et al. 2021) that capture atmospheric moisture of the humid winds coming from the Pacific Ocean. These fog oases are known to sustain species-rich floras (Rundel et al. 1991) and faunas of epigean beetles (Pizarro-Araya et al. 2023), suggesting the potential presence of endogean species of beetles and other arthropods that will require future focussed studies.

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The following colleague received images of the herein reported specimens and offered taxonomic opinions: Corylophidae: Richard A. B. Leschen (Auckland, New Zealand); Curculionidae: Cyclominae: Robert S. Anderson (Ottawa, Canada; he also advised us about a specimen of Chilean Raymondionyminae in CMN), and Juan. J. Morrone (Mexico City, Mexico); Eupsilobiidae: Emmanuel Arriaga-Varela (Warsaw, Poland); Leiodidae: Alfred F. Newton (Chicago, IL, USA); Ptiliidae: Alfred F. Newton and Mikael Sörensson (Lund, Sweden); Scarabaeidae: Showtaro Kakizoe (Tokyo, Japan) and José Mondaca (Valparaíso, Chile); Staphylinidae (unless otherwise noted below): Alfred F. Newton; Staphylinidae: Euaesthetinae: David J. Clarke (Memphis, USA); Staphylinidae: Pselaphinae: Donald S. Chandler (Durham, NH, USA), Peter Hlaváč (Prague, Czech Republic), and Giorgio Sabella (Palermo, Italy); Staphylinidae: Scydmaeninae: Paweł Jałoszyński (Wroclaw, Poland); Tenebrionidae: Erich L. Spiessberger (Tübingen, Germany) and Michael A. Ivie (Bozeman, MT, USA); Zopheridae: Michael A. Ivie. Additionally, colleagues offered their opinions after seeing pictures of the unidentified member of the superfamily Elateroidea (Fig. 2): Ladislav Bocak, Dominik Kusy, and Michal Motyka (all: Olomouc, Czech Republic), Hume Douglas (Ottawa, Canada), Frank Etzler (Helena, MT, USA), Matthew L. Gimmel (Santa Barbara, CA, USA), Michael A. Ivie, John F. Lawrence (Gympie, Australia), Richard A.B. Leschen, and Wioletta Tomaszewska (Warsaw, Poland).

Keith Hubbard (Ottawa, Canada) helped with SEM imaging. CA was funded through the Ramón y Cajal program by the AEI (Spain/FEDER, EU, ID RyC2021-034291-I)."

6. Supplementary online material

File 1. 50 deep soil samples taken in Chile. Here we provide 50 photographs, one for each among 50 Chilean spots where deep soil samples were taken, illustrating the sample volume and surrounding vegetation. A quick look through these images will give an adequate impression of the Chilean habitats supporting endogean beetles.

File 2. 2,037 endogean beetles collected in Chile. These 197 photographs document every endogean beetle specimen we collected. Each photograph shows all specimens from an individual sample belonging to one among 19 taxonomic units (as in Table 1).

File 3. 190 specimens of Chilean endogean beetles selected for DNA barcode. Each specimen is shown in two views (hence 380 high-magnification photographs).

7. References

Andújar, C., P. Arribas, F. Ruzicka, A. Crampton-Platt, M.J.T.N. Timmermans & A. Vogler (2015): Phylogenetic community ecology of soil biodiversity using mitochondrial metagenomics. – Molecular Ecology **24**: 3603–3617 [https://doi.org/10.1111/mec.13195].

Andújar, C., A. Faille, S. Pérez-González, J. P. Zaballos, A. P. Vogler & I. Ribera (2016): Gondwanian relicts and oceanic dispersal in a cosmopolitan radiation of euedaphic ground beetles. – Molecular Phylogenetics and Evolution **99**: 235–246 [https://doi.org/10.1016/j.ympev.2016.03.013].

Andújar, C., P. Hlaváč & V. V. Grebennikov (2024): Mitochondrial phylogenomics reveals the sister relationship between the endogean Mediterranean raymondionymine weevils and the remaining 51,000+ Curculionidae (Coleoptera). – Arthropod Systematics & Phylogeny XX: XXX–XXX [https://doi.org/10.3897/asp.XXXX].

Andújar, C. & V. V. Grebennikov (2021): Endogean beetles (Coleoptera) of Madagascar: deep soil sampling and illustrated overview. – Zootaxa **4963**: 317–334 [https://doi.org/10.11646/zootaxa.4963.2.4].

Arribas, P., C. Andújar, A. Salces-Castellano, B. C. Emerson & A. P. Vogler (2021): The limited spatial scale of dispersal in soil arthropods revealed with whole-community haplotype-level metabarcoding. – Molecular Ecology **30**: 48–61 [https://doi.org/10.1111/mec.15591].

Asenjo, A., U. Irmler, J. Klimaszewski, D. S. Chandler, H. E. Fierros-López & J. S. Vieira (2019): Staphylinidae (Insecta: Coleoptera) in Latin America: synopsis, annotated catalog, diversity and distribution. – Zootaxa **4621**: 1–406 [https://doi.org/10.11646/zootaxa.4621.1.1].

Austin, A., M. Guzik, K. Jones, W. Humphreys, C. Watts & S. J. B. Cooper (2023): The unique Australian subterranean Dytiscidae: diversity, biology, and evolution. – In: Yee, D. A. (ed.): Ecology, Systematics, and the Natural History of Predaceous Diving Beetles (Coleoptera: Dytiscidae). – Springer, Chasm: 401–425 [https://doi.org/10.1007/978-3-031-01245-7_9].

Baehr, M. (2014): Three new species and a new genus of Zuphiini from the Pilbara, north-western Australia (Insecta: Coleoptera: Carabidae: Zuphiini). – Records of the Western Australian Museum **29**: 95–104 [https://doi.org/10.18195/issn.0312-3162.29(2).2014.095-104].

Barrios-Izás, M. A., C. Andújar & V. V. Grebennikov (2024): Endogean beetles (Coleoptera) of Guatemala: deep soil sampling and illustrated DNA barcode library. – Soil Organisms **96**: 1–9 [https://doi.org/10.25674/349].

Bekchiev, R. & B. Guéorguiev (2014): First purposive study of beetles (Coleoptera) from endogean environments in Bulgaria: collection sites and preliminary results. – Biodiversity Journal **5**: 327–340.

Baviera, C., C. Bellò & G. Osella (2012): First record of the genus *Bordoniola* Osella, 1987 in Ecuador with description of five new species (Coleoptera: Curculionidae, Raymondionyminae). – Zootaxa **3455**: 69–80.

Bocak, L., R. Kundata, C. Andujar Fernandez & A. P. Vogler (2016): The discovery of Iberobaeniidae (Coleoptera: Elateroidea): a new family of beetles from Spain, with immatures detected by environmental DNA sequencing. – Proceedings of the Royal Society B: Biological Sciences **283**, 20152350: 7 pp. [https://doi.org/10.1098/rspb.2015.2350].

Bonniard de Saludo, P. (1969): Nouveaux carabiques du Chili. – Bulletin de la Société d'Histoire Naturelle de Toulouse **105**(3-4): 311–328.

Caterino, M. S. & E. Recuero (2024): Shedding light on dark taxa in sky-island Appalachian leaf litter: Assessing patterns of endemicity using large-scale, voucher-based barcoding. – Insect Conservation and Diversity **17**: 16–30 [https://doi.org/10.1111/icad.12697].

Cicchino, A. C. & S. Roig-Juñent (2001): Description and relationships of *Paranillopsis* new genus, two new species from Argentina, and a key to the Neotropical genera of the subtribe Anillina (Coleoptera: Carabidae: Bembidiini). – The Coleopterists Bulletin **55**: 185–193 [https://doi.org/10.1649/0010-065X(2001)055[0185:DAROPN]2.0.CO;2].

Coiffait, H. (1958): Les coléoptères du sol. Supplément numéro 7 à Vie et Milieu. Bulletin du Laboratoire Aragó. Actualités scientifiques et industrielles 1260. – Editions scientifiques Hermann, Paris, 204 pp.

Douglas, H. B., R. Kundrata, A. J. Brunke, H. E. Escalona, J. T. Chapados, J. Eyres, R. Richter, K. Savard, A. Ślipiński, D. McKenna & J. R. Dettman (2021) Anchored phylogenomics, evolution and systematics of Elateridae: Are all bioluminescent Elateroidea derived click beetles? – Biology **10**(6) 451: 25 pp. [https://doi.org/10.3390/biology10060451].

Drumont, A., H. Raemdonck, L. Dahan, M. Delbol & A. Kuhn A. (2020): Note sur la présence en Belgique de *Ferreria marqueti* (Aubé, 1863) (Insecta, Coleoptera, Curculionidae). – Le Coléoptériste **23**: 19–23.

Elgueta, M. & A. E. Marvaldi (2006): Lista sistemática de las especies de Curculionoidea (Insecta: Coleoptera) presentes en Chile, con su sinonímia. – Boletín del Museo Nacional de Historia Natural **55**: 113–153.

Fancello, L., C. Hernando & P. Leo (2009): The endogean beetle fauna of the Marganai-Oridda-Valle del Leni area (SW Sardinia), with description of seven new species of Staphylinidae Leptotyphlinae (Coleoptera). – Zootaxa **2318**: 317–338.

Folmer, O., M. Black, W. Hoeh, R. Lutz & R. Vrijenhoek (1994): DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. – Molecular Marine Biology and Biotechnology **3**: 294–299.

García, R., C. Andújar, P. Oromí, B. C. Emerson & H. López H. (2019): The discovery of *Barretonus* (Curculionidae: Cossoninae) in the Canary Islands: barcoding, morphology and description of new species. – Acta Entomologica Musei Nationalis Pragae **59**: 443–452 [https://doi.org/10.2478/aemnp-2019-0033].

Giachino, P. M., S. Eberhard & G. Perina (2021): A rich fauna of subterranean short-range endemic Anillini (Coleoptera, Carabidae, Trechinae) from semi-arid regions of Western Australia. – ZooKeys **1044**: 269–337 [https://doi.org/10.3897/zookeys.1044.58844].

Giachino, P. M. & D. Vailati (2010): The subterranean environment. Hypogean life, concepts and collecting techniques. L'ambiente sotterraneo. Vita ipogea, concetti e tecniche di raccolta. WBA Handbooks 3. – World Biodiversity Association, Verona: 130 pp.

Grebennikov, V. V. & A. F. Newton (2008): Minute larvae of Leptotyphlinae (Coleoptera: Staphylinidae): description of three genera with discussion on the monophyly and phylogenetic position of the subfamily as inferred from larval morphology. – Zootaxa **1817**: 49–58.

Guzik, M. T., A. D. Austin, S. J. B. Cooper, M. S. Harvey, W. F. Humphreys, T. Bradford, S. M. Eberhard, R. A. King, R. Leys, K. A. Muirhead & M. Tomlinson (2011): Is the Australian subterranean fauna uniquely diverse? – Invertebrate Systematics **24**: 407–418 [https://doi.org/10.1071/IS10038].

Hartley, A. J., G. Chong, J. Houston & A. E. Mather (2005): 150 million years of climatic stability: evidence from the Atacama Desert, northern Chile. – Journal of the Geological Society **162**: 421–424 [https://doi.org/10.1144/0016-764904-071].

Hebert, P. D. N., S. Ratnasingham & J. R. DeWaard (2003): Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species. – Proceedings of the Royal Society B: Biological Sciences **270**: 96–99 [http://dx.doi.org/10.1098/rsbl.2003.0025].

Herman, L. H. (2001a): Catalog of the Staphylinidae (Insecta: Coleoptera). 1758 to the end of the second millennium. III. Oxyteline Group. – Bulletin of the American Museum of Natural History **265**: 1067–1806.

Herman, L. H. (2001b): Catalog of the Staphylinidae (Insecta: Coleoptera). 1758 to the end of the second millennium. IV. Staphylinine Group (Part 1). – Bulletin of the American Museum of Natural History **265**: 1807–2440.

Howden, A. T. (1992): Review of the New World eyeless weevils with uncinate tibiae (Coleoptera, Curculionidae; Molytinae, Cryptorhynchinae, Cossoninae). – Memoirs of the Entomological Society of Canada **162**: 1–76 [https://doi.org/10.4039/entm124162fv].

Hu, F.-S., E. Arriaga-Varela, G. Biffi, L. Bocák, P. Bulirsch, A. F. Damaška, J. Frisch, J. Hájek, P. Hlaváč, B.-H. Ho

& et al. (2024): Forest leaf litter beetles of Taiwan: first DNA barcodes and first insight into the fauna. – Deutsche Entomologische Zeitschrift **71**: 17–47 [https://doi.org/10.3897/dez.71.112278].

Irmler, U. (2001): New Neotropical species of the genera *Clavilispinus*, *Aneucamptus*, *Thoracophorus*, and *Holotrochus* (Coleoptera: Staphylinidae, Osoriinae). – Amazoniana **16**: 349–361.

Irmler, U. (2016): A new genus and new species of Neotropical Thoracophorini (Coleoptera: staphylinidae: Osoriinae). – Bonn Zoological Bulletin **64**(2): 109–116.

Ivie, M.A., N. P. Lord, I. A. Foley & S. A. Ślipiński (2016): Colydiine genera (Coleoptera: Zopheridae: Colydiinae) of the New World: a key and nomenclatural acts 30 years in the making. – The Coleopterists Bulletin **70**: 755–788 [https://doi.org/10.1649/0010-065X-70.4.755].

Jałoszyński, P. & A. F. Newton (2017): Taxonomy of *'Euconnus* complex'. Part XV. Disentangling the *Euconnus-Sciacharis* knot (Coleoptera, Staphylinidae, Scydmaeninae). – Zootaxa **4344**: 401–443 [https://doi.org/10.11646/zootaxa.4344.3.1].

Jamieson, A.J. & J. N. J. Weston (2023): Amphipoda from depths exceeding 6,000 meters revisited 60 years on. – Journal of Crustacean Biology **43**: 1–28 [https://doi.org/10.1093/jcbiol/ruad020].

Jeannel, R. (1962a): Les Psélaphides de la Paléantarctide occidentale. – In: Deboutteville, C. D. & E. Rapoport (eds): Biologie de l'Amérique Australe. Vol. I. – Etudes sur la Faune du Sol. – Centre National de la Recherche Scientifique, Paris: 295–479.

Jeannel, R. (1962b): Les Trechides de la Paléantarctide occidentale. – In: Deboutteville, C. D. & E. Rapoport (eds): Biologie de l'Amérique Australe. Vol. I. – Etudes sur la Faune du Sol. – Centre National de la Recherche Scientifique, Paris: 527–655.

Leschen, R. A. B. (1996): Phylogeny and revision of the genera of Cryptophagidae (Coleoptera: Cucujoidea). – The University of Kansas Science Bulletin **55**: 549–634.

Magrini, P. & A. Benelli (2018): Note sul genere *Perucharidius* Mateu & Etonti, 2002 con descrizione di una nuova specie (Coleoptera, Carabidae, Anillina). – Giornale Italiano di Entomologia **15**(63): 63–74.

Mateu, J. & M. Etonti (2002): *Perucharidius andinus* gen. n., sp. n. di Anillini del Peru settentrionale (Coleoptera: Carabidae: Anillini). – Atti del Museo Civico di Storia Naturale, Trieste **49**: 129–132.

Miller, M., W. Pfeiffer & T. Schwartz (2010): Creating the CIPRES Science Gateway for inference of large phylogenetic trees. – 2010 Gateway Computing Environments Workshop, New Orleans, Louisiana 2010: 1–8 [https://doi.org/10.1109/GCE.2010.5676129].

Moat, J., A. Orellana-Garcia, C. Tovar, M. Arakaki, C. Arana, A. Cano, L. Faundez, M. Gardner, M., Hechenleitner, M., J. Hepp & et al. (2021): Seeing through the clouds – mapping desert fog oasis ecosystems using 20 years of MODIS imagery over Peru and Chile. – International Journal of Applied Earth Observations and Geoinfmation **103**, 102468: 13 pp. [https://doi.org/10.1016/j.jag.2021.102468]

Morrone, J. J. (2013): The subtribes and genera of the tribe Listroderini (Coleoptera, Curculionidae, Cyclominae): Phylogenetic analysis with systematic and biogeographical accounts. – ZooKeys **273**: 15–71 [https://doi.org/10.3897/zookeys.273.4116].

Morrone, J. J., & R. S. Anderson (1995): The *Falklandius* generic group: Cladistic analysis with description of new taxa (Coleoptera, Curculionidae, Rhytirrhinini). – American Museum Novitates 3121: 1–14 [http://hdl.handle.net/2246/3563].

Muona, J., H. Chang & D. Ren (2020): The clicking Elateroidea from Chinese Mesozoic deposits (Insecta, Coleoptera). – Insects **11**, 875: 16 pp. [https://doi.org/10.3390/insects11120875].

Newton, A. F. (1985): South temperate Staphylinoidea (Coleoptera): their potential for biogeographic analysis of austral disjunctions. – In: Ball, G. E. (ed.): Taxonomy, phylogeny and zoogeography of beetles and ants. – Series Entomologica **33**. – W. Junk, Dordrecht: 180–220.

Newton, A. F. (1998): Phylogenetic problems, current classification and generic catalogue of world Leiodidae (including Cholevinae). In: Phylogeny and evolution of subterranean and endogean Cholevidae (=Leiodidae Cholevinae), proceedings of XX international congress of Entomology, Firenze, 1996. – Atti del Museo regionale di Scienze naturali, Torino: 41–178.

Newton, A. F. (2000): The turtle Leiodid, a Chilean enigma. – Abstracts book II, XXIth International Congress of Entomology, Foz do Iguassu: 935 p.

Nishikawa, M. (2018): *Muganryus susumui* gen. et sp. nov. (Coleoptera, Leiodidae, Catopocerinae) from Hokkaido, a beetle subfamily new for the Japanese fauna. – Elytra, New Series **8**(2): 407–416.

Olberg, S. & K. M. Olsen (2009) The genus *Anommatus* Wesmael, 1835 (Coleoptera, Bothrideridae) in Norway. – Norwegian Journal of Entomology **56**: 143–145.

Pakaluk, J. & S. A. Ślipiński (1990): Review of Eupsilobiinae (Coleoptera: Endomychidae) with descriptions of new genera and species from South America. – Revue Suisse de Zoologie **97**: 705–728.

Parra-Gómez, A. & L. D. Fernández (2022): Filling gaps in the diversity and biogeography of Chilean millipedes (Myriapoda: Diplopoda). – Arthropod Systematics & Phylogeny **80**: 561–573 [https://doi.org/10.3897/asp.80.e86810].

Pérez-Delgado, A. J., P. Arribas, C. Hernando, L. López, Y. Arjona, D. Suárez-Ramos, B. C. Emerson & C. Andújar (2022): Hidden island endemic species and their implications for cryptic speciation within soil arthropods. – Journal of Biogeography **49**: 1367–1380 [https://doi.org/10.1111/jbi.14388].

Peck, S. B. & J. Cook (2011): Systematics, distributions and bionomics of the Catopocerini (eyeless soil fungivore beetles) of North America (Coleoptera: Leiodidae: Catopocerinae). – Zootaxa **3077**: 1–118.

Perreaut, M. (2019): *Archaeocerus uenoi* n. gen. n. sp. (Coleoptera Leiodidae Catopocerinae) from Albian/Cenomanian age amber of Myanmar. – Zootaxa **4638**: 595–600 [https://doi.org/10.11646/zootaxa.4638.4.9].

Perreau, M. & J. Růžička (2007): Systematic position of *Perkovskius* Lafer 1989 (Coleoptera: Leiodidae), with description of a second species from Far East of Russia. – Annales de la Société entomologique de France (Nouvelle Série) **43**: 257–264 [https://doi.org/10.1080/00379271.2007.10697521].

Pizarro-Araya, J., E. V. Villalobos, F. M. Alfaro & A. Moreira-Muñoz (2023): Conservation efforts in need of survey improvement in epigean beetles from the Atacama coast, Chile. – Journal of Arid Environments **214**, 104995: 8 pp. [https://doi.org/10.1016/j.jaridenv.2023.104995].

Rambaut, A. (2020): FigTree. Version 1.4.4. Program and documentation. Available from: http://tree.bio.ed.ac.uk/software/figtree/ (accessed 19 April 2024).

Ratnasingham, S. & P. D. N. Hebert (2007): BOLD: the barcode of life data system. – Molecular Ecology Notes **7**: 355–364 [https://doi.org/10.1111/j.1471-8286.2007.01678.x].

Ratnasingham, S. & P. D. N. Hebert (2013): A DNA-based registry for all animal species: the barcode index number (BIN) system. – PLoS ONE **8** (8), e66213: 1–16 [https://doi.org/10.1371/journal.pone.0066213].

Robertson, J. A., A. Ślipiński, K. Hiatt, K. B. Miller, M. F. Whiting & J. V. McHugh (2012): Molecules, morphology and minute hooded beetles: a phylogenetic study with implications for the evolution and classification of Corylophidae (Coleoptera: Cucujoidea). – Systematic Entomology **38**: 209–232 [https://doi.org/10.1111/j.1365-3113.2012.00655.x].

Roig-Juñent, S. & M. Domínguez (2001): Diversidad de la familia Carabidae (Coleoptera) en Chile. – Revista Chilena de Historia Natural **74**: 549–571 [https://doi.org/10.4067/S0716-078X2001000300006].

Rosa, S. P., C. Costa, K. Kramp & R. Kundrata (2021): Hidden diversity in the Brazilian Atlantic rainforest: The discovery of Jurasaidae, a new beetle family (Coleoptera, Elateroidea) with neotenic females. – Scientific Reports **10**, 1544: 12 pp. [https://doi.org/10.1038/s41598-020-58416-6].

Rundel, P., W., M. O. Dillon, B. Palma, H. A. Mooney, S. L. Gulmon & J. R. Ehleringer (1991): The phytogeography and ecology of the coastal Atacama and Peruvian deserts. – Aliso **13**: 1–49 [https://doi.org/10.5642/aliso.19911301.02].

Sáiz, F. (1973): Un nouveau *Geomitopsis* du Chili (Col. Staphylinidae). – Nouvelle Revue d'Entomologie **3**: 19–21.

Sáiz, F. (1974) Revisión de la subfamilia Leptotyphlinae (Col. Staph.) en Chile, con notas sobre du ecologia y su biogeografia. (II Contribución). – Revista Chilena de Entomología **8**: 47–66.

Shokralla, S., T. M. Porter, J. F. Gibson, R. Dobosz, D. H. Janzen, W. Hallwachs, G. B. Golding & M. Hajibabaei (2015): Massively parallel multiplex DNA sequencing for specimen identification using an Illumina MiSeq platform. – Scientific Reports **5**, 9687: 7 pp. [https://doi.org/10.1038/srep09687].

Spiessberger E. L. & M. A. Ivie (2020): A new genus and fourteen new species of Anopidiina (Coleoptera: Tenebrionidae: Diaperinae: Gnathidiini) from the West Indies. – The Coleopterists Bulletin **74**: 667–695 [https://doi.org/10.1649/0010-065X-74.4.667].

Stamatakis, A. (2014): RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. – Bioinformatics **30**: 1312–1313 [https://doi.org/10.1093/bioinformatics/btu033].

Stebnicka, Z.T., M. Dellacasa & P. E. Skelley (2003): Review of New World Aegialiini (Coleoptera: Scarabaeidae: Aphodiinae), with descriptions of two new genera from South America. – Insecta Mundi **17**: 73–83.

Versluijs, R., M. Geertsma, R. Felix, H. Turin & T. van Noordwijk (2013): Eerste vondst van de blinde loopkever *Anillus caecus* in Nederland (Coleoptera: Carabidae). – Entomologische Berichten **185**: 185–190.

Vidal, P. & M. Guerrero (2007): Los Tenebriónidos de Chile. – Ediciones Universidad Católica de Chile, Santiago: 478 pp.

von Beeren, C., S. Pohl, M. Fikaček, S. Kleinfelder, A. K. Tishechkin, S. Yamamoto, M. Chani-Posse, D. Żyła, A. Tokareva, M. Maruyama & et al. (2023): Army ant middens — home and nursery of a diverse beetle fauna. – Ecology and Evolution **13**, e10451: 18 pp.

Yu, D.W., Y. Ji, B. C. Emerson, X. Wang, C. Ye, C. Yang & Z. Ding (2012): Biodiversity soup: Metabarcoding of arthropods for rapid biodiversity assessment and biomonitoring. – Methods in Ecology and Evolution **3**(4): 613–623 [https://doi.org/10.1111/j.2041-210X.2012.00198.x].