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A new species of the highly polytypic South American rodent *Ctenomys* increases the diversity of the *magellanicus* clade

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Abstract

The subterranean rodent *Ctenomys* is the most polytypic South American mammal genus and one of the most speciose and rapidly diversifying mammal genera in the world. Its systematics is unstable due to the underlying accelerated diversification processes that give rise to evolutionary lineages at different stages of differentiation and to remarkable morphological homogeneity even among long-differentiated species. As a result, species boundaries are often difficult to define. Diversity of this genus in the coastal area of central Argentina has been extensively studied, with two independent lineages currently recognized while a distinct third population had not been previously detected. Through a phylogenetic analysis based on combined morphological and molecular evidence, Bayesian estimates of divergence times, and morphometric and morphological assessments, we recognize this third population as an independently evolving lineage. The new species, *Ctenomys pulcer* **sp. nov.**, is here described for both the living fauna and the fossil record of the Pampean region of central Argentina. According to phylogenetic results, *Ctenomys pulcer* **sp. nov.** belongs to the essentially Patagonian *magellanicus* clade, and would have diverged from its sister species, *Ctenomys bidaui*, during the middle Pleistocene (ca. 0.4 Ma). Its current distribution in the fixed and semifixed dunes of the coastal Pampean region is assumed to represent a relict of a wider and continuous distribution of potentially suitable environments during the late Pleistocene. *Ctenomys pulcer* **sp. nov.** occurs in a particularly fragile natural system subjected to profound disturbances caused by diverse anthropic actions and therefore measures for the conservation of its habitat will be indispensable.

Keywords

Ctenomyidae, early Holocene-Recent, Pampean region, phylogeny, systematics

Introduction

The subterranean rodent *Ctenomys* is the most polytypic South American mammal genus and one of the most speciose and rapidly diversifying mammal genera in the world (Mammal Diversity Database 2022). The genus is distributed from the Peruvian highlands, at approximately 15° S latitude, to southernmost Argentina and Chile, occupying very diverse habitats from deserts to wooded areas, and from sea level to 5,000 m a.s.l. in the Andean range (Bidau 2015; Freitas 2016). Currently, 66 species are recognized as formally described independent units (Bidau 2015; Freitas 2016; D'Elía et al. 2021; Brook et al. 2022; Mapelli et al. 2022), and several other populations have been or are being recognized as separately evolving lineages, although they have not yet been formally nominated. As pointed out by D'Elía et al. (2021), the systematics of *Ctenomys* remains in a state of flux due to both intrinsic aspects of its evolutionary process and the patchy history of its study. Concerning the first factor, the very recent and accelerated diversification process of *Ctenomys* (De Santi et al. 2021; Upham et al. 2021) has resulted in evolving populations that are at different stages of morphological, genetic and/or ecological differentiation (review in Freitas 2021). Thus, species boundaries may often be difficult to define. In addition, remarkable morphological homogeneity is frequent even among long-standing differentiated species (D'Elía et al. 2021). In such a still largely inconclusive scenario, however, major systematic and phylogenetic knowledge has been generated in just over two decades (e.g., Lessa and Cook 1998; D'Elía et al. 1999; Mascheretti et al. 2000; Slamovits et al. 2001; Castillo et al. 2005; Parada et al. 2011; Gardner et al. 2014; Bidau 2015; Freitas 2016, 2021; Caraballo and Rossi 2018; Fornel et al. 2018; Leipnitz et al. 2020; Teta and D'Elía 2020; D'Elía et al. 2021; De Santi et al. 2021). One of the most significant advances is the recognition of informal groups of species based on increasingly well-supported clades (Parada et al. 2011; Freitas et al. 2012; Gardner et al. 2014; Caraballo and Rossi 2018; Londoño-Gaviria et al. 2018; Leipnitz et al. 2020; De Santi et al. 2020, 2021; Carnovale et al. 2021).

Because advances in the systematics of the genus *Ctenomys* have encompassed its diversity in a sectorized and disparate manner, the current taxonomic knowledge of *Ctenomys* remains uneven among species groups and geographic areas. While for some species or clades, their alpha taxonomy, phylogeny, and geographic variation are relatively well studied, others are known only through original descriptions, which are often insufficient for making taxonomic decisions (see reviews in Freitas 2021 and D'Elía et al. 2021). In this context, the populations of *Ctenomys* distributed in the coastal area of central Argentina have been extensively studied through different approaches and disciplines, including alpha taxonomy and morphology (Thomas 1898, 1912; Rusconi 1934; Contreras and Reig 1965; Reig et al. 1965, 1990; Contreras 1972; Vitullo et al. 1988; De Santis et al. 1998; Zenuto et al. 2003; Justo et al. 2003; Medina et al. 2007; García Esponda et al. 2009), cytogenetics and genetics (Kiblisky and Reig 1966; Massarini et al. 1991, 1995, 2002; Cutrera et al. 2005), phylogeography (Mora et al. 2006, 2010), ecology and behavior (Contreras and Reig 1965; Pearson et al. 1968; Antinuchi and Busch 1992; Zenuto and Busch 1995; Zenuto and Fanjul 2002; Busch et al. 2000; Justo et al. 2003; Schleich and Busch 2002a, 2002b; Cutrera et al. 2006; Antinuchi et al. 2007; Vassallo 2006), functional morphology (Vassallo 1998; Echeverría et al. 2017), among others. These studies were focused on the two species of *Ctenomys* recognized for this area, i.e., *Ctenomys talarum* and *Ctenomys australis*. In 1997, a population of a species distinct from the two abovementioned ones was detected by one of us (DHV) at the Atlantic coastal region, in the area of Monte Hermoso; its recognition was based on a specimen (MLP-Mz 24.IX.69.1) housed at the Mammal Collection of the Museo de La Plata. Subsequently, three field trips to the area during 1998 and 1999 allowed us to document the presence of this population in environments not occupied by either *C. talarum* or *C. australis*. In addition, the study of fossils collected at the Monte Hermoso I site (Massoia 1988; Politis and Bayón 1995; Bayón and Politis 1996; Pardiñas 2001) also allowed us to detect the presence of this lineage of *Ctenomys* in the late Pleistocene–early Holocene record of the area (De Santi et al. 2018). Although this lineage was studied through cytogenetic (Massarini et al. 1995), phylogeographic (Mora et al. 2007), and paleontological approaches (Massoia 1988; Pardiñas 2001), the aforementioned morphological homogeneity of *Ctenomys* conspired against its recognition as an entity distinct from *C. talarum*. Moreover, when preliminarily recognized as a morphologically distinct entity (informally as *Ctenomys* "monte", Morgan 2009; Morgan and Verzi 2006, 2011; Morgan et al. 2017 or *Ctenomys* sp. C, Verzi et al. 2021; De Santi 2022), it was incorrectly assigned to the *mendocinus* species group (De Santi et al. 2021; Verzi et al. 2021).

Here we describe both the extant and fossil representatives of this lineage of *Ctenomys* as a new species and assess its phylogenetic position and divergence time; additionally, we discuss aspects of its evolutionary history.

Materials and Methods

Morphology of the living and fossil representatives of the new species was compared with that of 661 specimens belonging to 54 species of *Ctenomys* (Table S1). The studied materials are housed in the following collections: **CFA**, Fundación de Historia Natural Félix de Azara, Buenos Aires, Argentina; **CML**, Colección Mamíferos Lillo, Tucumán, Argentina; **CNP**, Colección de Mamíferos del Centro Nacional Patagónico; **LIEB-M**, Laboratorio de Investigaciones en Evolución y Biodiversidad; **MACN-Ma**, **MACN-Pv**, Museo Argentino de Ciencias Naturales "Bernardino Rivadavia", Buenos Aires, Argentina; **MLP-Mz**, Museo de La Plata, La Plata, Argentina;

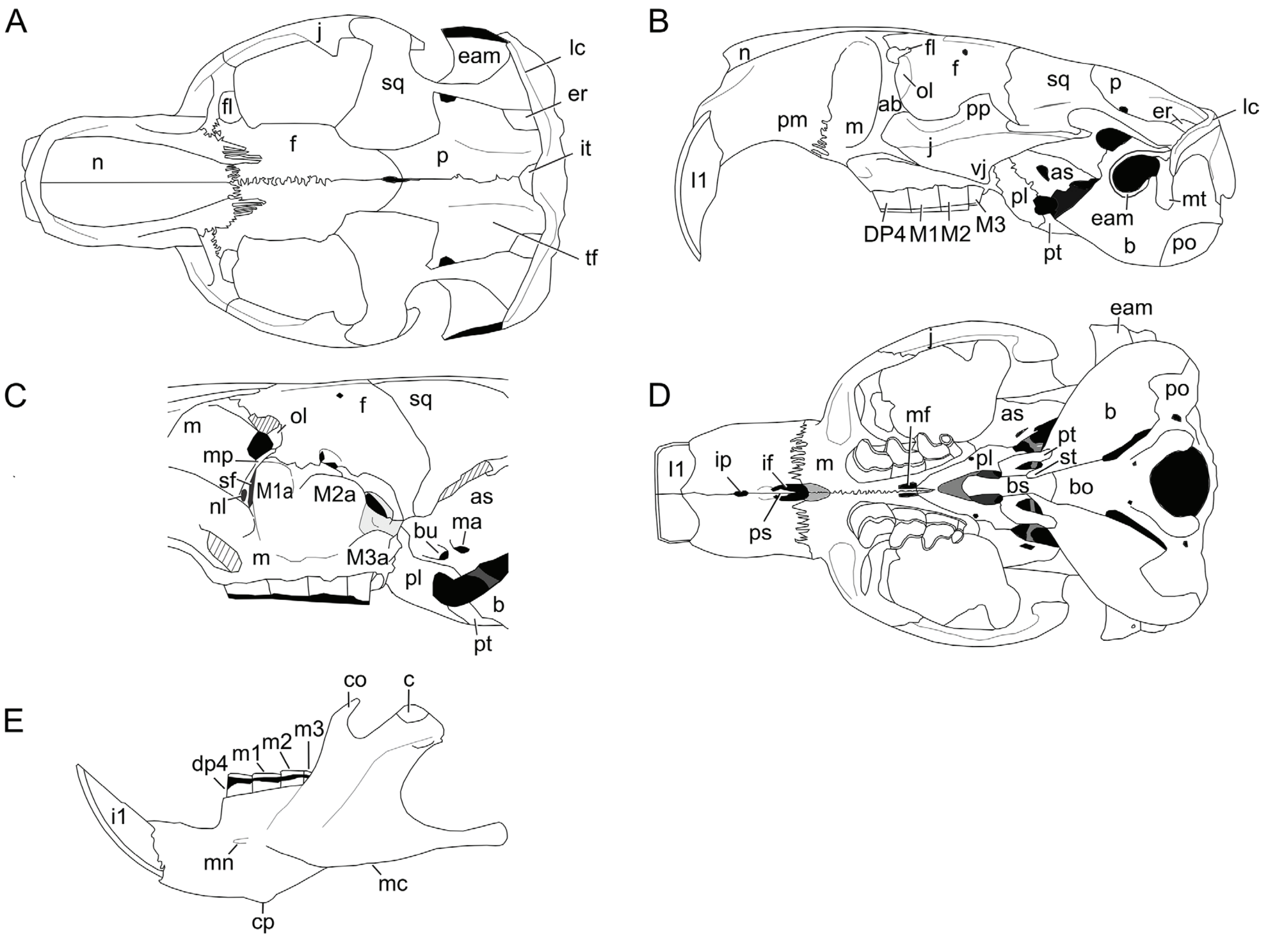

Figure 1. Nomenclature of craniomandibular traits (modified from Brook et al. 2022). Abbreviations: ab, antorbital bar; as, alisphenoid; b, auditory bulla; bo, basioccipital; bs, basisphenoid; bu, buccinator foramen; c, condyle; co, coronoid process; cp, chin process; dp4, lower deciduous premolar; DP4, upper deciduous premolar; eam, external auditory meatus; er, petrosal epitympanic recess; f, frontal; fl, facial portion of lacrimal; I1, upper incisor; i1, lower incisor; if, incisive foramen; ip, interpremaxillary foramen; it, interparietal; j, jugal; lc, lambdoid crest; m, maxilla; ma, masticatory foramen; mc, masseteric crest; mf, major palatine foramen; mn, mandibular notch; mp, maxillary plate; mt, mastoid apophysis; m1-m3, lower molars; M1-M3, upper molars; n, nasal; nl, foramen into nasolacrimal canal; ol, orbital portion of lacrimal; p, parietal; pl, palatine; pm, premaxilla; po, paraoccipital process; pp, paraorbitary process; ps, premaxillary septum; pt, pterygoid; sf, sphenopalatine fissure; sq, squamosal; st, styliform process; tf, temporal fossa; vj, ventral jugal process.

MMH, Museo Municipal de Ciencias Naturales "Vicente Di Martino", Monte Hermoso, Argentina, **MMP-Ma**, Museo de Ciencias Naturales de Mar del Plata "Lorenzo Scaglia", Mar del Plata, Argentina; **UACH**, Colección de Mamíferos de la Universidad Austral de Chile, Valdivia, Chile (M, Ma, Mz, Mammal collection; Pv, Vertebrate Paleontology collection). Nomenclature of craniomandibular traits is shown in Fig. 1. Fifteen cranial, mandibular and dental measurements were taken from each specimen: **BL**, basilar length; **BCL**, basioccipital length; **ZW**, maximum bizygomatic width; **CIL**, condyle–incisive length; **CL**, condylar length; **DL**, upper diastema length; **IB**, distance between anterior margin of mandibular foramen (mf) and extreme tip of condyle (estimates depth of insertion of the lower incisor; Verzi and Olivares 2006); **IW**, incisors width; **UTL**, upper toothrow length; **ZL**, length of the zygomatic arch; **MW**, maximum mandibular width; **Proc**, upper incisor procumbency as expressed by Thomas' angle (Reig et al. 1965); **RW**, width of the rostrum at level of premaxilla–maxilla suture; **LTL**, lower toothrow length; **ZI**, zygomatic index is the ratio of the length between the antorbital zygomatic bar and the paraorbitary process (x) divided by the length between the paraorbitary process and the posterior end of the zygomatic arch (y); ZI is an estimator of orbital size. All measurements were taken using a digital caliper (0.01 mm) and are expressed in millimeters except for procumbency (Proc) expressed in degrees. Standard external measures include **TBL**, total body length; **TL**, tail length; **HF**, hind foot length (including the claw); **EL**, ear length; **W**, weight (in grams).

DNA extraction, amplification and sequencing

Genomic DNA was extracted from tissues preserved in alcohol using the phenol/chloroform protocol (Sambrook and Russell 2001). Partial PCR amplification of cytochrome *b* (cyt *b*) and cytochrome oxidase I (COI) were obtained for one individual (MLP-Mz 13.VI.02.1) of the new species of *Ctenomys* from Estancia Delta (38°56'47" S 61°15'22" W), Monte Hermoso, Buenos Aires, Argentina. The primers used were as follows: TUTU-F (5`-CCTTCATAGGCTACGTAC-3`) / TUTU-R (5`- CTTCATTTGAGTAGTTTAT-3`) for cyt *b*, and LCO1490 (5`- GGTCAACAAATCATAAAGATATTGG-3`) / HCO2198 (5`- TAAACTTCAGGGTGACCAAAAAATCA-3`) for COI (Folmer et al. 1994; Caraballo et al. 2012). The PCR products were purified with Geneclean III (MP Biomedicals) and sequenced on an ABI 3130 capillary genetic analyzer (Applied Biosystems, Inc.). Sequencing reactions were performed following the standard protocol for Big Dye Terminators v3.1 (Applied Biosystems) in both directions, using DNABaser v. 3 (Heracle BioSoft, Pitesti, Romania) for contigs. The Genbank accession numbers for the new sequences are OP797665 (COI) and OP795710 (cyt *b*) (Table S2).

Phylogeny

A parsimony analysis was performed to assess the phylogenetic relationships of living and fossil samples of the new species and other 54 living species of the genus representing all the informally recognized species groups (Parada et al. 2011; D'Elía et al. 2021), plus two extinct species known from well-preserved cranial and mandibular remains. The morphological dataset including forty-eight characters correspond to that defined by De Santi et al. (2021) for the crown group of *Ctenomys*. In addition, we included a new character (49; Table S3) referred to the morphology of the ventral spine of the styliform process of the auditory bulla. According to the systematic arrangement proposed by Teta and D'Elía (2020) and D'Elía et al. (2021), we consider *C. colburni* as a junior synonym of *C. magellanicus* and thus included it in the phylogenetic analyses as *C. magellanicus* from Lago Buenos Aires County (Santa Cruz Province, Argentina). Likewise, we consider *C. coyhaiquensis* to be a junior synonym of *C. sericeus* and included it in phylogenetic analyses as *C. sericeus* from Chile Chico (Aysén, Chile). The echimyid *Thrichomys laurentius*, and the octodontids *Octomys mimax* and *Octodontomys gliroides* were included as outgroups. The analysis was performed based on the combined data set of 49 morphological characters and 11 gene fragments: five mitochondrial genes that encode for 12S ribosomal subunit (12S), cytochrome *b* protein (cyt *b*), cytochrome c oxidase subunit I (COI), cytochrome oxidase subunit II (COII), control region, and six nuclear genes including growth hormone receptor (GHR), interphotoreceptor retinoid-binding protein (IRBP), melanocortin receptor 1 (MC1R), recombination activating gene 1 (RAG1), transthyretin gene (TTH) and von Willebrand factor (vWF). Accession numbers of sequences are listed in Table S2. Except for cyt *b*, taxa sampling was partial for the remaining ten genes analyzed (Table S2). The sequences were previously aligned automatically using ClustalW (Thompson et al. 1994) in BioEdit 7.0.5.3 (Hall 1999). The combined matrix contained a total of 9,054 characters and 64 terminals. All characters were considered as equally weighted, and multistate characters were coded as nonadditive. The software TNT v.1.5 (Goloboff et al. 2008a, 2008b; Goloboff and Catalano 2016) was used to find the most parsimonious trees and assess branch support. The heuristic search for the most parsimonious trees consisted of 10,000 random stepwise–addition replicates and tree bisection reconnection (TBR) branch swapping, saving 100 trees per replicate. In addition, we performed an extra round of TBR on the optimal trees to increase the chance of finding all minimum–length topologies (Bertelli and Giannini 2005). Zero–length branches were collapsed if they lacked support under any of the most parsimonious reconstructions (Coddington and Scharff 1994). Branch support was calculated in the form of absolute and relative Bremer indices (Bremer 1994; Goloboff and Farris 2001).

In addition, we assessed phylogenetic relationships and divergence times through Bayesian inference method employing a Birth-Death speciation model into a node-dating analysis. The gene sequences were those used in parsimony analysis. The alignment, editing and concatenation of sequences of the 11 genes into a unique matrix of 9,004 bp were performed using BioEdit 7.0.5.3 (Hall 1999). To determine the appropriate molecular evolution model for the genes considered, jModelTest 2.1.7 (Posada 2008) was run for each of them. The models of nucleotide substitution were GTR for 12S and vWF, and HKY for cyt *b*, COI, COII, Control Region (D-loop), GHR, IRBP, MC1R, RAG1, and TTH. The input file was built using BEAUti v.2.6.1 with the following settings: each gene was considered as an independent partition. We used a log normal relaxed molecular clock to model substitution rate variation among branches (Drummond et al. 2006; Drummond and Rambaut 2007). Three fossil calibrations were used for dating nodes (Table S4). All fossil constraints were set as minimum hard bounds and lognormal and gamma priors were used to set soft upper bounds. The analyses were performed using Markov Chain Monte Carlo (MCMC) simulations for five independent runs with 50,000,000 generations and a sample frequency of 5,000. Phylogenetic relationships and divergence time among taxa were estimated through Bayesian inference methods implemented in BEAST 2.3.1 (Drummond and Rambaut 2007; Suchard and Rambaut 2009). Finally, we computed the maximum credibility tree from the resulting trees of BEAST analysis with a burn–in of 20% in TreeAnnotator v2.6.0 (Drummond and Rambaut 2007).

Pairwise genetic distances (p-distance) between species of the *magellanicus* group were estimated with MEGA11 ignoring sites with missing data.

Geometric morphometrics

Variation in cranial shape was analyzed using 2D geometric morphometric techniques. For this analysis, we selected 62 crania belonging to adult and subadult individuals of the four species and populations that were most closely related to living (n = 19) and fossil (n = 1) representatives of the new species in the phylogenetic analyses (see results below and details in Table S1). Two-dimensional coordinates were captured from digital images of the ventral and lateral view of cranium. Twenty-four landmarks and 30 semilandmarks were used to capture the ventral cranial shape, and 28 landmarks and 27 semilandmarks for the lateral cranial shape (Table S5). Landmark coordinates were digitized using tpsDig version 2.26 (Rohlf 2016). The resulting Procrustes shape coordinates were analyzed using MorphoJ (Klingenberg 2011) through principal component analyses (PCA). Principal components summarize and describe the major trends in shape variation among species and facilitate the visualization of shape ordination in a low-dimensional morphospace.

Results

Systematics

Superfamily Octodontoidea Waterhouse, 1839

Family Ctenomyidae Lesson, 1842

Genus *Ctenomys* Blainville, 1826

Ctenomys pulcer sp. nov.

https://zoobank.org/D1C2FC17-0BE2-40BC-A85C-8872BF5A4C07

Ctenomys talarum – Massoia (1988: 6)
Ctenomys cf. *Ctenomys talarum* – Pardiñas (2001: 236, fig. 2c)
Ctenomys talarum – Massarini et al. (1995: 208, 211-212, fig. 4)
Ctenomys talarum – Mora et al. (2007: 3457, fig 2a)
Ctenomys sp. “monte” – Morgan and Verzi (2006: 1260)
Ctenomys “monte” – Morgan and Verzi (2011: 4)
Ctenomys sp. “monte” – Morgan et al. (2017: 121, fig. 2)
Ctenomys sp. – De Santi et al. (2018: 71)
Ctenomys sp. C – De Santi et al. (2021: 8, 10, figs 4, 5)
Ctenomys sp. C – Verzi et al. (2021: 5, fig. 1.1)

Holotype. MLP-Mz 8.X.02.17 male, collected by D. Verzi and E. Etcheverry leg. on 5 July 1999, and prepared as study skin and skeleton.

Type locality. Argentina, Buenos Aires Province, Monte Hermoso County, Estancia Delta (38°56’47”S; 61°15’22”W; Fig. 2).

Paratypes. 14 specimens from Estancia Delta, Monte Hermoso County, Buenos Aires Province, Argentina (MLP-Mz 27.XII.01.56 male, MLP-Mz 27.XII.01.57 male, MLP-Mz 27.XII.01.58 male, MLP-Mz 13.VI.02.1 male, MLP-Mz 13.VI.02.2 female, MLP-Mz 9.XII.02.1 female, MLP-Mz 9.XII.02.2 female, MLP-Mz 27.XII.01.48 female, MLP-Mz 27.XII.01.49 male, MLP-Mz 27.XII.01.54 female, MLP-Mz 27.XII.01.55 female, MLP-Mz 30.XII.02.17 male, MLP-Mz 2536 male, MLP-Mz 2537 female); three specimens from Sauce Grande lagoon, Monte Hermoso County, Buenos Aires Province, Argentina (38°56’51”S; 61°20’51”W; MLP-Mz 3.XII.02.14 male, MLP-Mz 2538 male, MLP-Mz 3027 male). See Table S1.

Other referred specimens. MLP-Mz 3.V.48.4, MLP-Mz 24.IX.69.1, MLP-Mz 18.XI.98.1, MLP-Mz 18.XI.98.2, MLP-Mz 27.XII.01.47, MLP-Mz 27.XII.01.50, MLP-Mz 3028, MLP-Mz 3029, MLP-Mz 3030; MMP-Ma 1776, MMP-Ma 1796, MMP-Ma 1804, MMP-Ma 1806, MMP-Ma 2584; MMH 3.85, MMH 84.2.2, MMH 86.3, MMH 86.3.2, MMH 86.3.3, MMH 86.3.4, MMH 88.2.4, MMH 88.2.5, MMH 89.2.5, MMH 89.2.7, MMH 89.12.2, MMH 89.12.4, MMH 89.12.5, MMH 89.12.7, MMH 89.12.8, MMH 90.1.5, MMH 90.1.10, MMH 90.2.1, MMH 90.2.13, MMH 90.2.14, MMH 91.9.5, MMH 91.9.8, MMH 91.9.10, MMH 92.11.8, MMH 92.11.11, MMH 92.11.18, MMH 92.11.19, MMH 93.11.4, MMH 93.11.5, MMH 94.12.2, MMH 94.12.3, MMH 94.12.5, MMH 94.12.6, MMH 95.11.2, MMH 95.11.4, MMH 95.11.5, MMH 95.11.8, MMH 96.12.5, MMH 96.12.8, MMH 96.12.10, MMH 96.12.11, MMH 97.9.2, MMH 97.9.3, MMH 97.9.6, MMH 97.11.4, MMH 97.11.8, MMH 98.10.2, MMH 98.10.3, MMH 98.10.4, MMH 98.10.5, MMH 98.10.8, MMH 98.12.1, MMH 98.12.2, MMH 98.12.4, MMH 99.10.4, MMH 99.10.6, MMH 99.10.7, MMH 99.10.10, MMH 99.10.11, MMH MH1, MMH MH3, MMH MH5, MMH MH6. See Table S1.

Diagnosis. A medium-sized species of *Ctenomys*. Coloration ochraceous with some orange on the dorsum, with a dark patch on the dorsal snout and head, and irregular dark zones along the middle of the back; paler toward the flanks, and buff yellowish ochre on the belly. Cranial rostrum with wide base due to divergent insertion of upper incisors. Incisive foramina short, and premaxillary septum wide. Medial margins of the premaxillaries flattened against the roots of the premaxillary septum. Facial portion of the lacrimal strongly reduced and not protruding; orbital portion of lacrimal interrupted by frontal. Alveolar sheath of M1 protruding into the lacrimal foramen, its anterodorsal portion covered by the maxillary plate posterior to the nasolacrimal canal. Edges of frontals posterior to the orbital constriction subparallel. Ventral spine of the styliform process of the auditory bulla long, especially in the living population. Tube of external auditory meatus long, especially its posterior wall. Bottom of alveolus of dp4 and m1 markedly protruding into the ventral portion of mandibular corpus. Masseteric crest subhorizontal.

Description and comparison. *Ctenomys pulcer* **sp. nov.** is a medium-sized *Ctenomys* (measurements in Table 1), slightly larger in size than its sister species *Ctenomys bidaui* (see below, Parsimony and Bayesian analyses). Pelage is dense, fine and silky. The dorsal coloration is ochraceous with some orange in most specimens (Fig. 3), with individual hairs buff yellowish to ochre at their bases and with dark brown to blackish tips. A more extended blackish section in individual hairs defines a dark patch

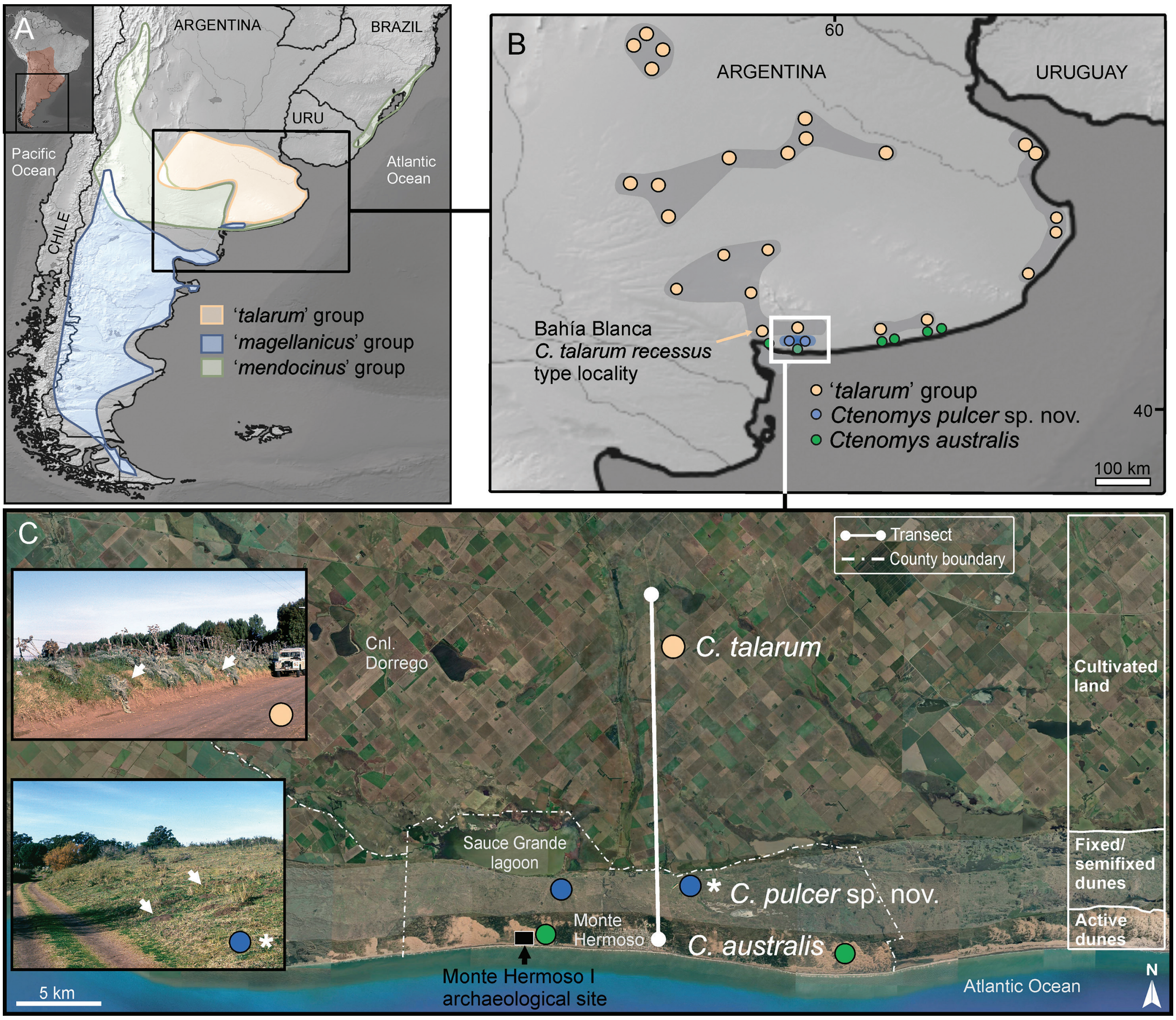

Figure 2. Geographical distribution of *Ctenomys pulcer*. **A** Geographical distribution of the *magellanicus*, *mendocinus* and *talarum* species groups (Bidau 2015; Tammone et al. 2022; Lacey et al. 2022); **B** distribution of *Ctenomys pulcer* **sp. nov.**, *talarum* group and *C. australis* in central-eastern Argentina (Bidau 2015; Carnovale et al. 2021; this work); **C** detail of southwestern coastal area of the Buenos Aires province displaying habitats and collecting sites of *C. pulcer* **sp. nov.** (blue dots), *C. talarum* (light orange dots), and *C. australis* (green dots) and the location of Monte Hermoso 1 site (black rectangle) [map data 2022 (C) Google]; the asterisk denotes Estancia Delta, the type locality of *Ctenomys pulcer* **sp. nov.**; white arrow in photographs denotes mounds. Photographs from July 1999.

on the dorsal snout and head, and irregular dark zones along the middle of the back. The coloration is paler toward the flanks, and buff yellowish ochre on the belly where individual hairs lack the dark tips. The coloration of the tail is pale ochre with a brownish dorsal section of variable development. Forelimb hairs and ungual bristles are whitish.

The cranium is similar in general shape to that of the sister species *C. bidaui*, but with a wider rostrum, smaller orbit, and anteriorly narrower auditory bullae (Figs 4 and 5). In the parapatric, similar-sized *C. talarum*, the rostrum is more expanded anteriorly, the zygomatic arches are more bowed, and the auditory bullae are less inflated (Fig. 4). The rostrum of *C. pulcer* has a wider base than that of *C. bidaui* because the incisor roots are more divergent. The interpremaxillary foramen is variable, well developed in some specimens and markedly reduced or absent in others (it is absent in the holotype MLP-Mz 8.X.02.17; Fig. 4A). In *C. bidaui* and *C. talarum* this foramen is always present and well developed. The premaxillary septum is wide, and the incisive foramina are slightly shorter and wider than those of *C. bidaui*, and shorter than those of *C. talarum*. The premaxillary anterior margins of the incisive foramina are dorso-medially convergent and flattened against the premaxillary septum. The palatal bridge, between the molar series, is wider than in *C. bidaui*. The major palatine foramina open on the maxillary at the level of M1, as in *C. bidaui*. The mesopterygoid fossa reaches the level of M2. The zygomatic arches are less bowed than those of *C. bidaui*. The postglenoid articular region (sensu Verzi and Olivares 2006), between the posterior border of the glenoid fossa and the external auditory meatus, is wider than that of *C. bidaui*, similar to that of *C. talarum* (Fig. 4). The auditory bullae are more inflated than those

Table 1. External and craniodental measurements (mm) of *Ctenomys pulcer* sp. nov. and *Ctenomys bidaui*. Abbreviations and descriptions are available in Materials and Methods. Standard external measurements of *C. bidaui* from Teta and D'Elía (2020).

	***Ctenomys pulcer* sp. nov. (holotype)**	***Ctenomys pulcer* sp. nov.**					***Ctenomys bidaui***				
		mean	**SD**	**min**	**max**	**n**	**mean**	**SD**	**min**	**max**	**n**
BL	35.69	32.32	2.69	29.70	37.56	20	32.06	2.31	29.51	35.72	5
BCL	7.94	7.09	0.75	6.24	8.82	20	7.07	0.54	6.39	7.84	5
ZW	24.66	23.23	1.92	21.49	27.39	19	23.02	2.04	21.38	26.54	5
ZL	19.31	17.95	1.07	16.32	19.76	20	17.41	0.89	16.39	18.98	6
RW	9.77	8.83	0.93	7.76	10.80	20	8.05	0.59	7.18	8.91	6
DL	10.76	9.86	1.39	8.35	13.20	20	10.19	0.87	8.92	11.45	6
UTL	8.32	8.22	0.79	7.26	9.87	20	7.93	0.34	7.49	8.41	6
ZI	0.92	0.93	0.10	0.75	1.09	19	1.20	0.11	1.10	1.40	6
IW	5.51	4.84	0.62	4.07	6.07	19	4.70	0.45	4.19	5.45	6
MW	32.56	29.71	3.01	26.88	34.88	16	29.02	1.96	27.16	32.13	5
LTL	9.34	8.70	0.65	7.80	10.00	20	8.30	0.42	7.88	8.81	5
CIL	30.75	28.49	2.61	25.95	33.40	19	28.51	1.41	26.88	30.68	5
IB	6.98	6.47	0.77	5.67	8.09	20	6.32	0.67	5.80	7.33	5
CL	3.7	2.71	0.32	2.07	3.30	20	3.19	0.20	2.94	3.46	5
Proc	97°	96°	4°	89°	102°	13	100°	2°	98°	101°	4
TBL	235	217.86	21.20	190.00	261.00	19	232.60	21.40	205.00	253.00	5
TL	70	67.50	7.37	55.50	81.00	19	64.30	9.70	53.00	73.20	5
HF	33	31.78	2.27	26.50	36.00	19	32.10	1.50	30.20	34.20	5
EL	7	6.65	0.70	6.00	8.00	19	7.10	0.90	6.30	8.30	5
W	170	123.35	41.83	85.00	225.00	19	132.80	24.80	105.00	165.00	5

Figure 3. Skins in dorsal and ventral view of *Ctenomys pulcer* **sp. nov. A** MLP-Mz 8.X.02.17 (holotype, male); **B** MLP-Mz 3027 (paratype, male); *Ctenomys bidaui* (**C**) CFA-MA 11867 (holotype, male). Scale bar 20 mm.

of *C. talarum*; they have their anterolateral portion less inflated than in *C. bidaui*. At the anterior end of the bulla, the ectotympanic forms an apophysis where the pterygoid process contacts the bulla (Fig. 6). This structure is ventral to the styliform process and here we refer to it as the ventral spine of the styliform process. This ventral spine attains strong development in living *C. pulcer*, extending markedly forward which is unique among the analyzed *Ctenomys* (Fig. 6). In the fossil †*C. pulcer* and especially in *C. bidaui* this apophysis is shorter. The external audi-

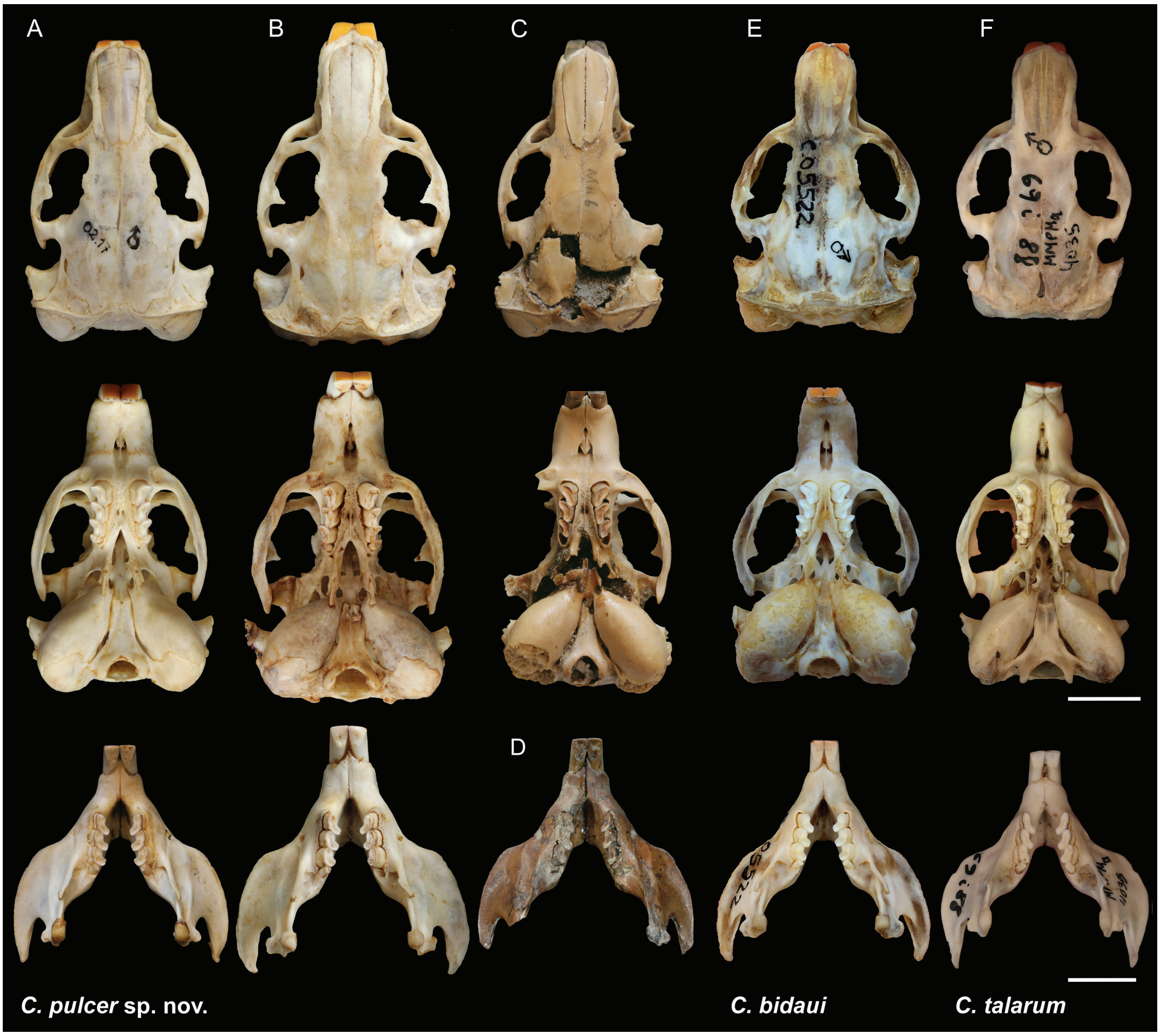

Figure 4. General morphology of the cranium (dorsal and ventral view) and mandible (dorsal view) of *Ctenomys pulcer* **sp. nov. A** MLP-Mz 8.X.02.17 (holotype, male); **B** MLP-Mz 3027 (paratype, male); **C** MMH6; **D** MMH3 (**C** and **D** fossil specimens); *Ctenomys bidaui* (**E**) CFA-MA 11867 (holotype, male); *Ctenomys talarum* (**F**) MMP-Ma 4035 (male). Scale bar 10 mm.

tory meatus forms a more protruding tube than that of *C. bidaui*; especially the posterior wall of this tube is clearly longer than in *C. bidaui* (Figs 4 and 7). In lateral view, the cranial roof is slightly vaulted (Fig. 5), with the nasal bone more noticeably arched in some specimens as MLP 9.XII.02.1 and MLP 9.XI.02.2. The zygomatic arch is dorsoventrally low due to the poor development of the paraorbital process and the ventral jugal process (Fig. 5); this is a characteristic shared mainly with species of the *mendocinus* and *magellanicus* species groups. The antorbital bar is slightly more oblique, antero-dorsally, than that of *C. bidaui*, in which it is more vertical. The facial portion of the lacrimal bone is very small (Fig. 8), similar to that of *C. australis*. The orbital portion of this bone is restricted to the dorsal portion of the nasolacrimal canal by the interposition of the frontal bone (Fig. 9). Similar to *C. bidaui*, the first part of the nasolacrimal canal is short because the foramen into the nasolacrimal canal is close to the facial portion of the lacrimal bone (Fig. 9). Comprehensive information on lacrimal variability in *Ctenomys* is not available, but at least in the sample analyzed, the morphology of this bone did not show relevant intraspecific variation. As indicated by the zygomatic index, the orbit of *C. pulcer* is smaller than that of *C. bidaui* (Fig. 5; ZI in Table 1). In the medial wall of the orbit, the maxillary plate delimiting the sphenopalatine fissure covers the anterodorsal portion of the M1 alveolar sheath, as is also the case in species of the *mendocinus* species group and *C. talarum* (Fig. 9). This M1 alveolar sheath is high in relation to the level of the facial portion of lacrimal bone. In *C. bidaui*, the anterodorsal portion of the alveolar sheath of M1 is exposed (Fig. 9). Similar to *C. bidaui*, the buccinator and masticatory foramina on the alisphenoid are mostly separated; however, this configuration is variable and in some specimens these foramina are merged into a single foramen. Both the palatine and pterygoid contact the auditory bulla, as in the other species of the *magellanicus* group and the species of the *mendocinus* group. The mastoid apophysis is short; its tip does not exceed the ventral margin of the external auditory meatus. Dorsally,

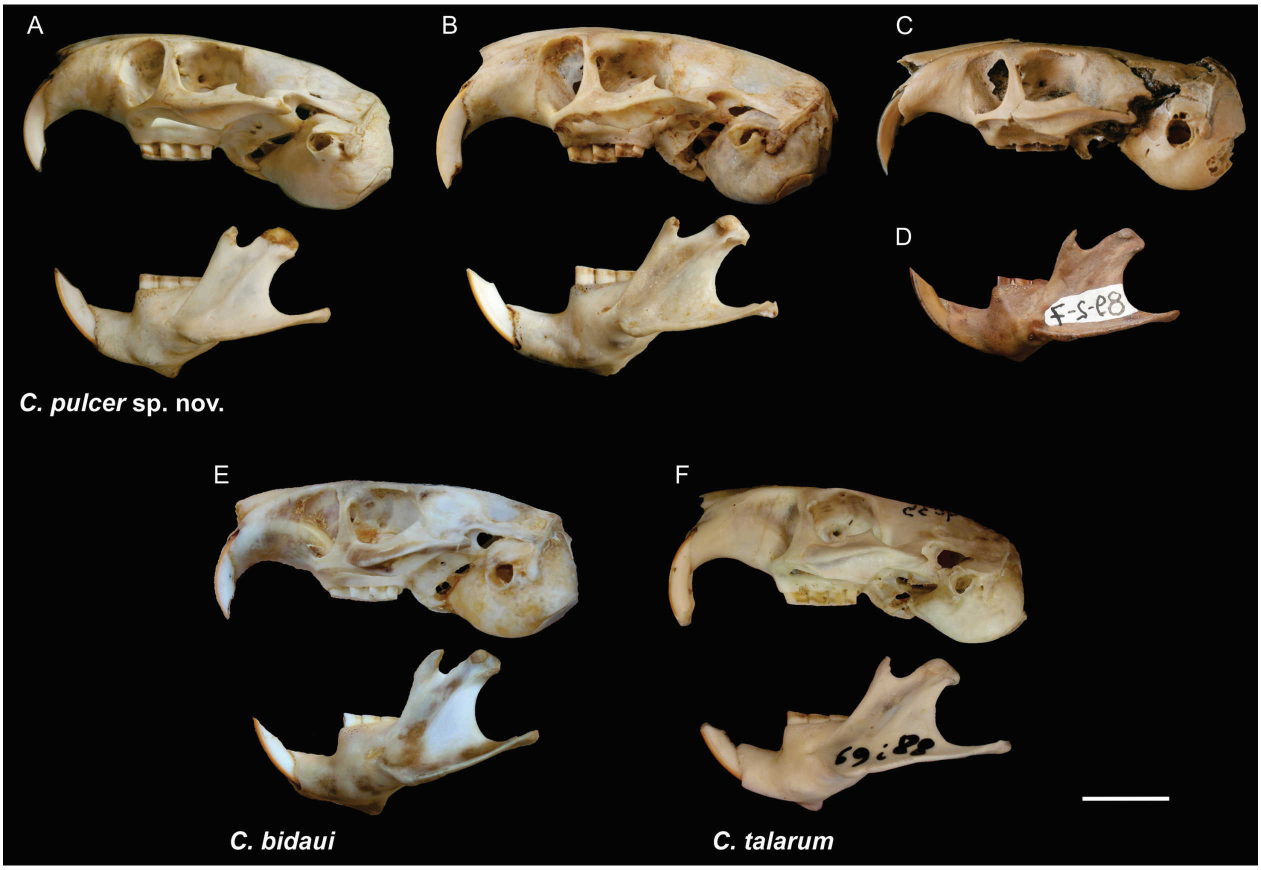

Figure 5. General morphology of the cranium and mandible in lateral view of *Ctenomys pulcer* **sp. nov. A** MLP-Mz 8.X.02.17 (holotype, male); **B** MLP-Mz 3027 (paratype, male); **C** MMH6; **D** MMH 89.2.7 (**C** and **D** fossil specimens; D reversed); **E** *Ctenomys bidaui*, CFA-MA 11867 (holotype, male); **F** *Ctenomys talarum*, MMP-Ma 4035 (male). Scale bar 10 mm.

the frontal margins of the orbits are subparallel, whereas these are more divergent in *C. bidaui* (Fig. 4). The temporal fossae are shallow as those of *C. bidaui*. A persistent interfrontal fontanelle is present in the holotype. There is an interparietal in adults discernible as a very short and wide ossification, similar to that of *C. bidaui*. The petrosal epitympanic recesses are small as in *C. bidaui* and wider than those of *C. talarum*. The lambdoid crest is slightly more developed than that of *C. bidaui*.

As in the other species of the crown group of *Ctenomys*, *C. pulcer* has a markedly hystricognathous mandible. In dorsal view, the masseteric crest is more expanded with respect to the mandibular corpus than in *C. bidaui*. The origin of the masseteric crest is posteroventral to the mandibular notch (for the insertion of the tendon of the infraorbital part of the medial masseter muscle). In lateral view, this origin of the masseteric crest is more separated from the mandibular notch than in *C. bidaui*. The masseteric crest is subhorizontal in *C. pulcer*; in *C. bidaui* this crest is more ascending and oriented in the same direction as the ventral margin of the mandibular body (Fig. 5). Anterolaterally to the origin of the crest, the bottoms of the alveolar sheath of dp4 and m1 form two protuberances on the lower margin of the mandibular corpus in *C. pulcer*, which are more protruding than those of *C. bidaui* (Fig. 5). The tip of the coronoid process reaches the level of the condylar process or is below it. The chin process is well developed and located at the level of the m1.

The upper incisors have divergent insertion. They are orthodont to slightly proodont as in *C. bidaui* (see Proc in Table 1). The lower incisors are not inserted deeply; the bottom of their alveolar sheath and associated mandibular foramen are located further away from the condyle than in more specialized tooth-digger species such as *C. leucodon*, *C. lewisi*, or *C. steinbachi* (Verzi and Olivares 2006; Morgan et al. 2017). The DP4–M3/dp4–m3 crowns lack cement.

Sperm morphology. Simple asymmetric (Fig. S1).

Karyotype. Massarini et al. (1995) described the karyotype and C-banding pattern of *C. talarum recessus* from Monte Hermoso. Revision of the vouchers, MMP-Ma 1776, MMP-Ma 1796, MMP-Ma 1804, MMP-Ma 1806, and MMP-Ma 2584 suggests that the 2N = 48 karyotype described by Massarini et al. (1995) corresponds to *C. pulcer* **sp. nov.**

Etymology. From Latin *pulcer*, in Spanish *hermoso* (beautiful) in reference to the type locality Monte Hermoso.

Distribution and habitat. Up to the present, *C. pulcer* **sp. nov.** has been found in Monte Hermoso County, in the southeastern Atlantic coast of Buenos Aires Province, in central Argentina. This area corresponds to the southern-

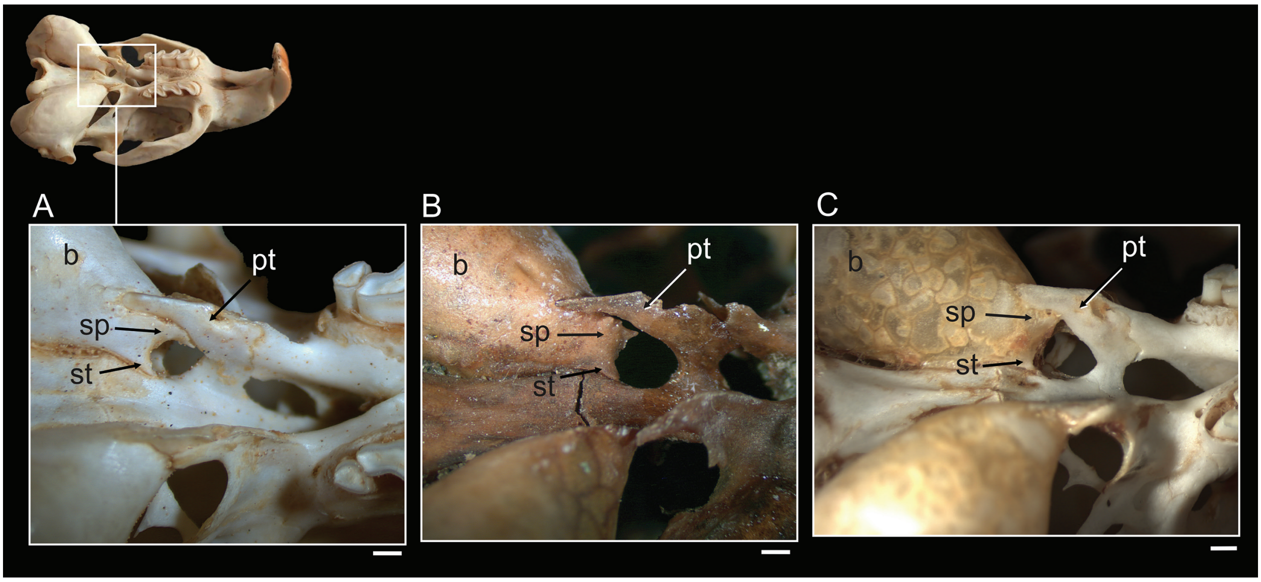

Figure 6. Anterior portion of auditory bulla in ventral view of *Ctenomys pulcer* **sp. nov. A** MLP-Mz 8.X.02.17 (holotype); **B** MMH 7-11-8 (fossil specimen); **C** *Ctenomys bidaui*, CFA-MA 11857. Abbreviations: b, auditory bulla (ectotympanic portion); st, styliform process; sp, ventral spine of the styliform process; pt, pterygoid. Scale bar 1 mm.

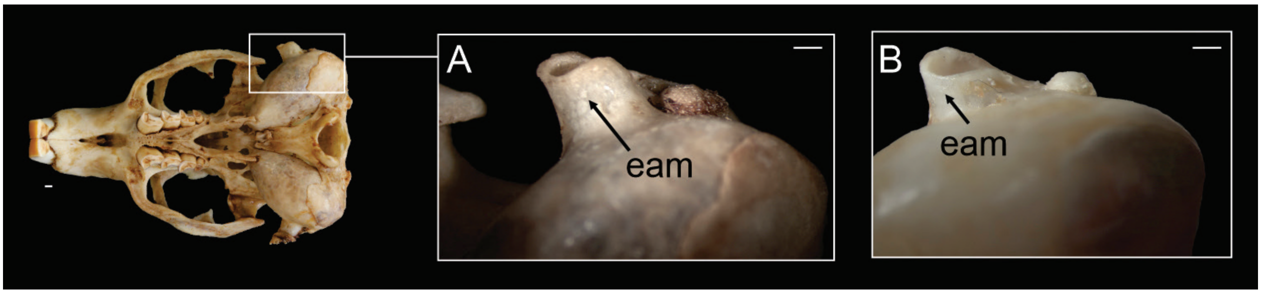

Figure 7. External auditory meatus (eam) in *Ctenomys pulcer* **sp. nov. A** MLP-Mz 3027; **B** *Ctenomys bidaui*, CFA-MA 11867 (holotype). Scale bar 1 mm.

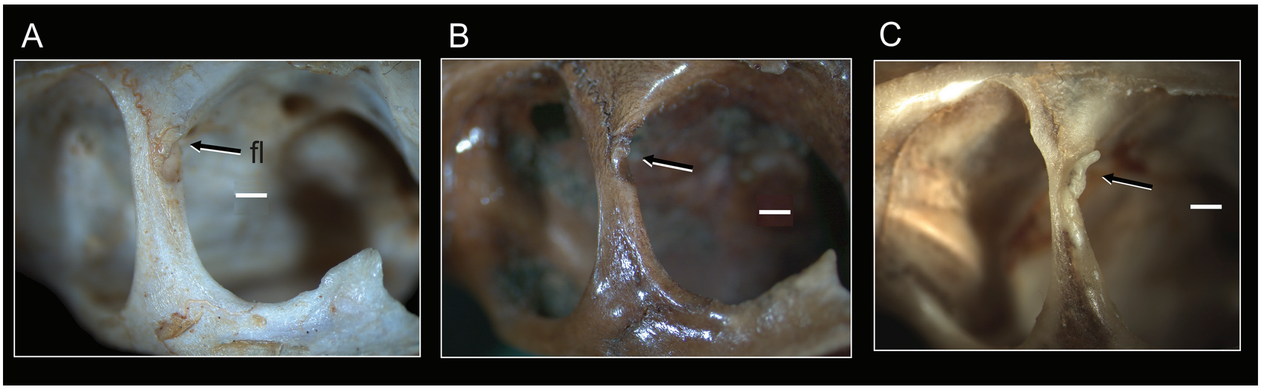

Figure 8. Facial portion of lacrimal in *Ctenomys pulcer* **sp. nov. A** MLP-Mz 8.X.02.17 (holotype); **B** MMH 7-11-8 (fossil specimen); **C** *Ctenomys bidaui*, CFA-MA 11857. Abbreviation: **fl** facial portion of lacrimal. Scale bar 1 mm.

most portion of the Pampean phytogeographic province and to the Austral Pampean district (Cabrera 1971, 1994). The predominant vegetation are grasslands that develop in a subhumid–dry climate, with a mean annual temperature of 15.4°C and mean annual precipitation of 684.9 mm (Cabrera 1971; Monserrat et al. 2012). In this area, *C. pulcer* is distributed in parapatry with *C. australis* and *C. talarum* (Fig. 2). Notably, the distribution pattern of the three species matches the three physiographic units recognized by Monserrat et al. (2012) on the basis of geomorphological characteristics and vegetation units; i.e., from shore to inland, *C. australis* occupies frontal active dunes, *C. pulcer* inhabits fixed/semifixed dunes, and *C. talarum* lives in more compact and humid soils at the edges of cultivated lands. This distribution was checked by conducting a 20 km shore-perpendicular

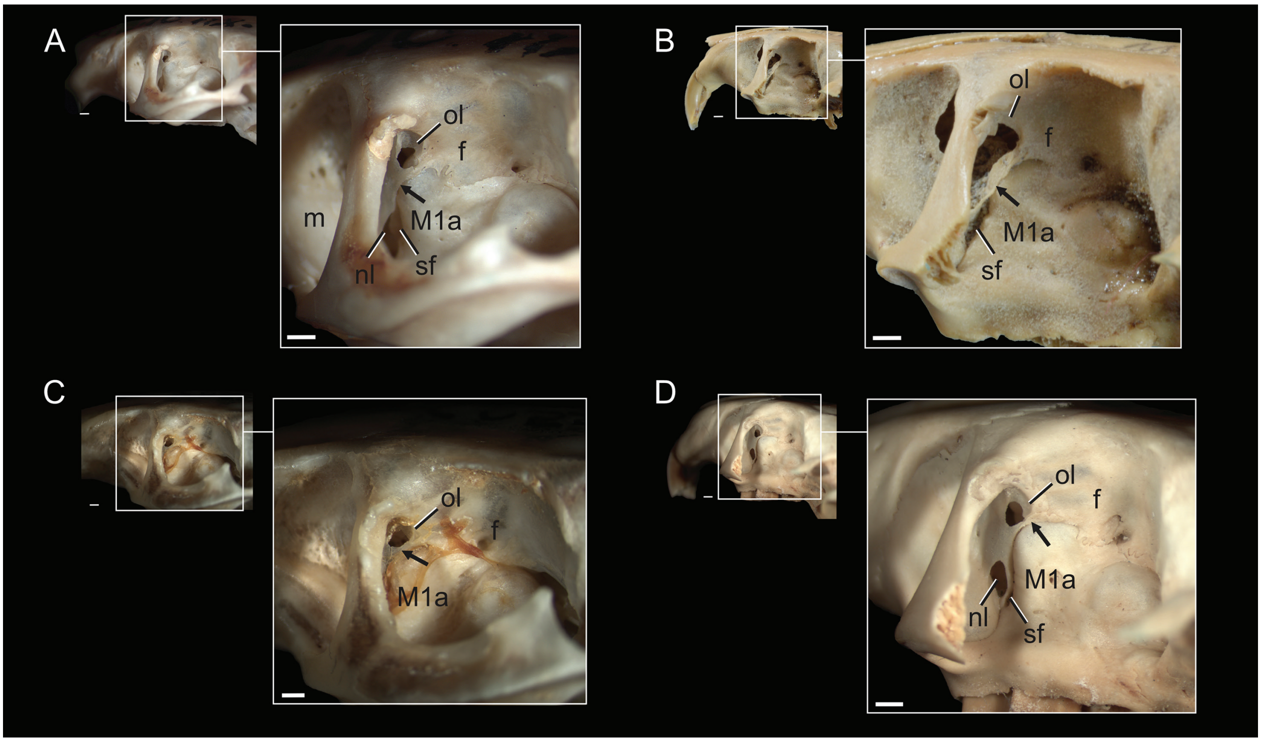

Figure 9. Orbital region in *Ctenomys pulcer* **sp. nov.** **A** MLP-Mz 27.XII.01.58; **B** MMH 6 (fossil specimen); *Ctenomys bidaui* (**C**) CFA-MA 11867 (holotype); **D** MLP 28.V.01.10. Black arrow indicates the maxillary plate. Abbreviations: f, frontal; m, maxilla; M1a, alveolar sheath of M1; nl, foramen into nasolacrimal canal; ol, orbital portion of lacrimal; sf, sphenopalatine fissure. Scale bar 1 mm.

transect (carried out by DHV and Eduardo Etcheverry, at midday in July 1999). *C. talarum* could be recognized by its vocalizations which are similar to those recorded in populations at the northern end of its distribution (DHV, pers. observ.); three specimens of this species were collected (MLP 2547, MLP 3.XII.02.15, MLP 3028). It is noteworthy that those populations of *C. talarum* distributed in southern coastal localities where *C. pulcer* is not present, occupy the fixed/semifixed dunes (Contreras and Reig 1965; Cutrera et al. 2006). This suggests that the segregation found in Monte Hermoso precludes competition of *C. talarum* with the larger-sized *C. pulcer* (see Vassallo 1993).

Natural history. At the two capture sites in Monte Hermoso area, Estancia Delta (38°56’47”S; 61°15’22”W) and the vicinity of Sauce Grande lagoon (38°56’51” S; 61°20’51”W) (Fig. 2), the population of *C. pulcer* **sp. nov.** was dense. In a sampling conducted on 5 July 1999 between 9.30 a.m. and 1 p.m. at the first station, seven animals were captured on a fixed dune extension of around 20 x 150 m. The dominant plant species in the fixed/semifixed dunes system inhabited by *C. pulcer* were *Poa lanuginosa*, *Panicum urvilleanum*, *Hyalis argentea*, *Sporolobus rigens*, and *Cortaderia selloana* (Celsi and Monserrat 2008a, 2008b; Monserrat 2010; Monserrat et al. 2012). Unlike *C. talarum*, the largest-sized individuals of *C. pulcer* were aggressive when removed from their burrows. Vocalizations were heard in the Sauce Grande lagoon station between 9.30 a.m. and 11 a.m.; these vocalizations are lower-pitched than those of *C. talarum*, and are composed of a series of tong-tong repeated without change of their frequency. A gravid female was recorded on 7 September 1998.

Fossil record. A rich fossil record of *Ctenomys* has been recovered from the current distribution area of *C. pulcer* **sp. nov.** (Figs 2, 10). The remains of *Ctenomys* were recovered during 1984 and 1985 by the late Vicente J. Di Martino at the site Monte Hermoso I (Figs 2, 10; Zavala et al. 1992; Politis and Bayón 1995; Bayón and Politis 1996). This site outcrops on the marine platform near the town of Monte Hermoso (37°57’47”S; 61°22’48”W). It consists of silty-sand deposits affected by marine abrasion (Zavala et al. 1992). Two units are recognized, a lower unit informally called “wackes inferiores” (lower wackes) and an upper unit informally called “pelitas grises” (gray pelites; Zavala et al. 1992). More recently, these sediments have been interpreted as part of the Agua Blanca Sequence (Zavala and Quattrocchio 2001; Zavala 2006). The “wackes inferiores” consist of massive grayish-green and dark olive wackes (see Zavala et al. 1992 for a more detailed description). Different datings have alternatively assigned this unit to the late Pleistocene (14,370 ± 60 BP; Bayón and Politis 1996) and to the early Holocene (8,990 ± 55 BP; based on collagen dating of a *Ctenomys* mandible; Pardiñas 2001). The upper unit of “pelitas grises” includes several overlying levels with human footprints dating from 7,920 to 6,600 BP (Bayón et al. 2011).

The fossil sample of †*C. pulcer* comprises 63 specimens from the “wackes inferiores.” This bearing deposit has yielded a rich vertebrate fauna (Massoia 1988; Pardiñas 2001) including the currently parapatric *C. australis* and *C. talarum* (Fig. 10; Table S1). The fossil sample

Table 2. Observed genetic distances (p-distances) of the cytochrome b gene between pairs of species and subspecies of *Ctenomys* of the '*magellanicus*' group. Abbreviations: CH, Chile Chico (Aysén, Chile); LB, Lago Buenos Aires County (Santa Cruz Province, Argentina).

	***C. magellanicus* LB**	***C. contrerasi contrerasi***	***C. contrerasi navoneae***	***C. sericeus* CH**	***C. haigi***	***C. magellanicus***	***C. pulcer* sp. nov.**	***C. sericeus***	***C. thalesi***
C. contrerasi contrerasi	0.048								
C. contrerasi navoneae	0.054	0.010							
***C. sericeus* CH**	0.049	0.020	0.025						
C. haigi	0.055	0.034	0.032	0.032					
C. magellanicus	0.004	0.049	0.057	0.052	0.057				
***C. pulcer* sp. nov.**	0.056	0.064	0.063	0.059	0.062	0.056			
C. sericeus	0.048	0.020	0.025	0.008	0.033	0.051	0.063		
C. thalesi	0.052	0.014	0.029	0.025	0.038	0.056	0.080	0.020	
C. bidaui	0.037	0.052	0.056	0.044	0.053	0.041	0.022	0.046	0.049

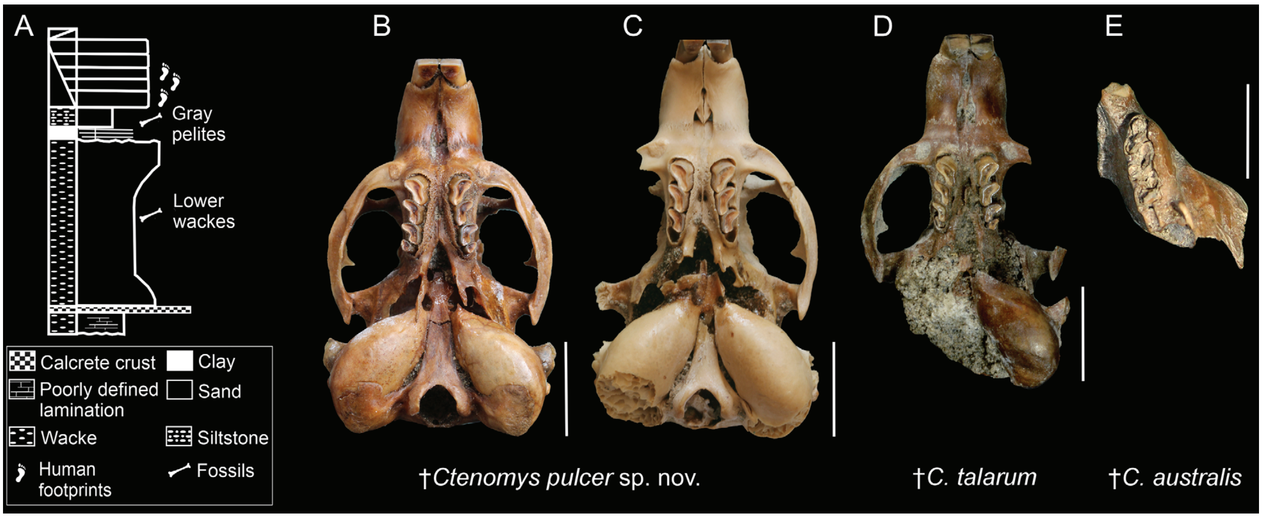

Figure 10. A Stratigraphical profile of the Monte Hermoso I archaeological site (modified from Zavala et. al 1992). **B**–**E** specimens recovered from this site; †*C. pulcer* **sp. nov.**: **B** MMH 94-11-8, **C** MH6 (crania in ventral view); **D** †*C. talarum* MMH 88.2.6 (cranium in ventral view); **E** †*C. australis* MH2 (right hemimandible in dorsal view). Scale bar 10 mm.

of †*C. pulcer* includes at least two well-preserved crania (MMH 97.11.8 and MMH MH6; Fig. 4C and Fig. 10B, C) and numerous rostral, palatal, and mandibular remains (Table S1). The cranial and mandibular morphology is in accord with that described for the living population. As an exception, the auditory bullae of fossil specimens present a slightly shorter ventral spine of the styliform process.

Specimens of †*C. pulcer* from the Monte Hermoso I site were assigned to *C. talarum* by Massoia (1988) and to *Ctenomys* cf. *talarum* by Pardiñas (2001). They were interpreted as an independent lineage belonging to the *mendocinus* species group by De Santi et al. (2018, 2021), Verzi et al. (2021), and De Santi (2022).

Parsimony analysis

The parsimony analysis based on the matrix of combined morphological and molecular data resulted in 16 most parsimonious trees, 3,879 steps long (CI = 0.58; RI = 0.60; Fig. 1). In the topology of the resulting strict consensus tree, the clade *C. sociabilis*–*C. plebiscitum* is sister to a clade comprising the rest of the species of the genus. In this latter major clade, the eight traditionally recognized informal species groups were recovered as monophyletic. The *frater* group is sister to a major clade formed by *C. tuconax*–*C. leucodon* and the species groups *tucumanus*–*magellanicus* and *boliviensis* (*opimus* (*torquatus* (*talarum*–*mendocinus*))). The *magellanicus*, *opimus*, *torquatus*, *talarum*, and *mendocinus* clades show no conflict (i.e., have high relative Bremer support) while the remaining species groups and major clades are poorly supported (Fig. 11). The Pleistocene †*C. subassentiens* is nested within the *frater* clade, forming a polytomy with *C. lewisi* and *C. frater*, while *C. osvaldoreigi*–†*C. viarapaensis*, here included only through morphology, are sisters to the *magellanicus* clade. The living and Holocene *Ctenomys pulcer* were recovered as nested within the *magellanicus* clade, forming a clade sister to *C. bidaui*. The observed genetic divergence (p-distance) between *Ctenomys pulcer* and *C. bidaui* was 0.022 (Table 2). The living and fossil populations from *C. pulcer*

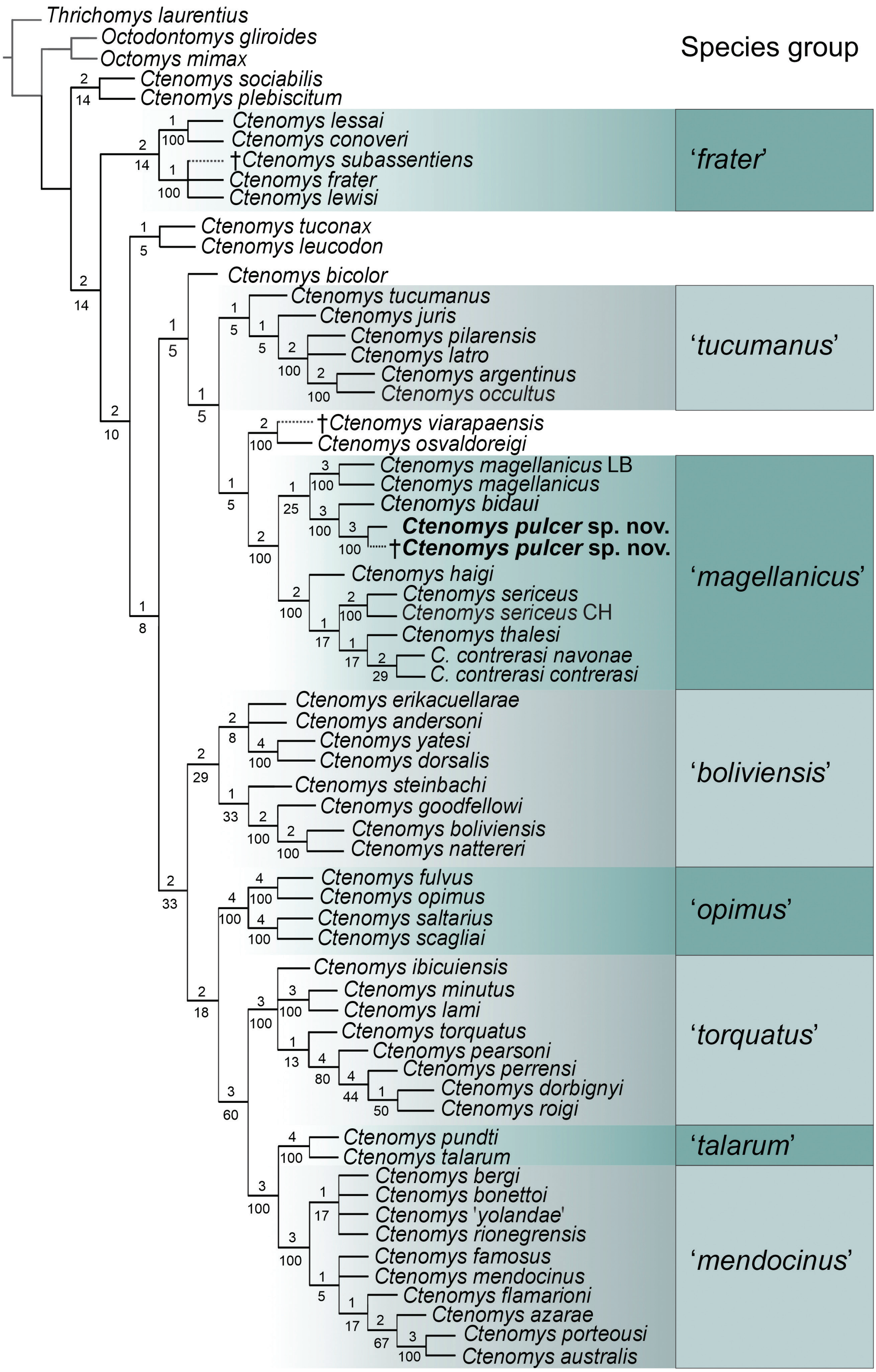

Figure 11. Strict consensus of 16 most parsimonious trees of 3,789 steps resulting from parsimony analysis of morphological and molecular data. Bremer support (above) and relative Bremer support values (below) are shown for each node. Abbreviations: **CH** Chile Chico (Aysén, Chile); **LB** Lago Buenos Aires County (Santa Cruz, Argentina).

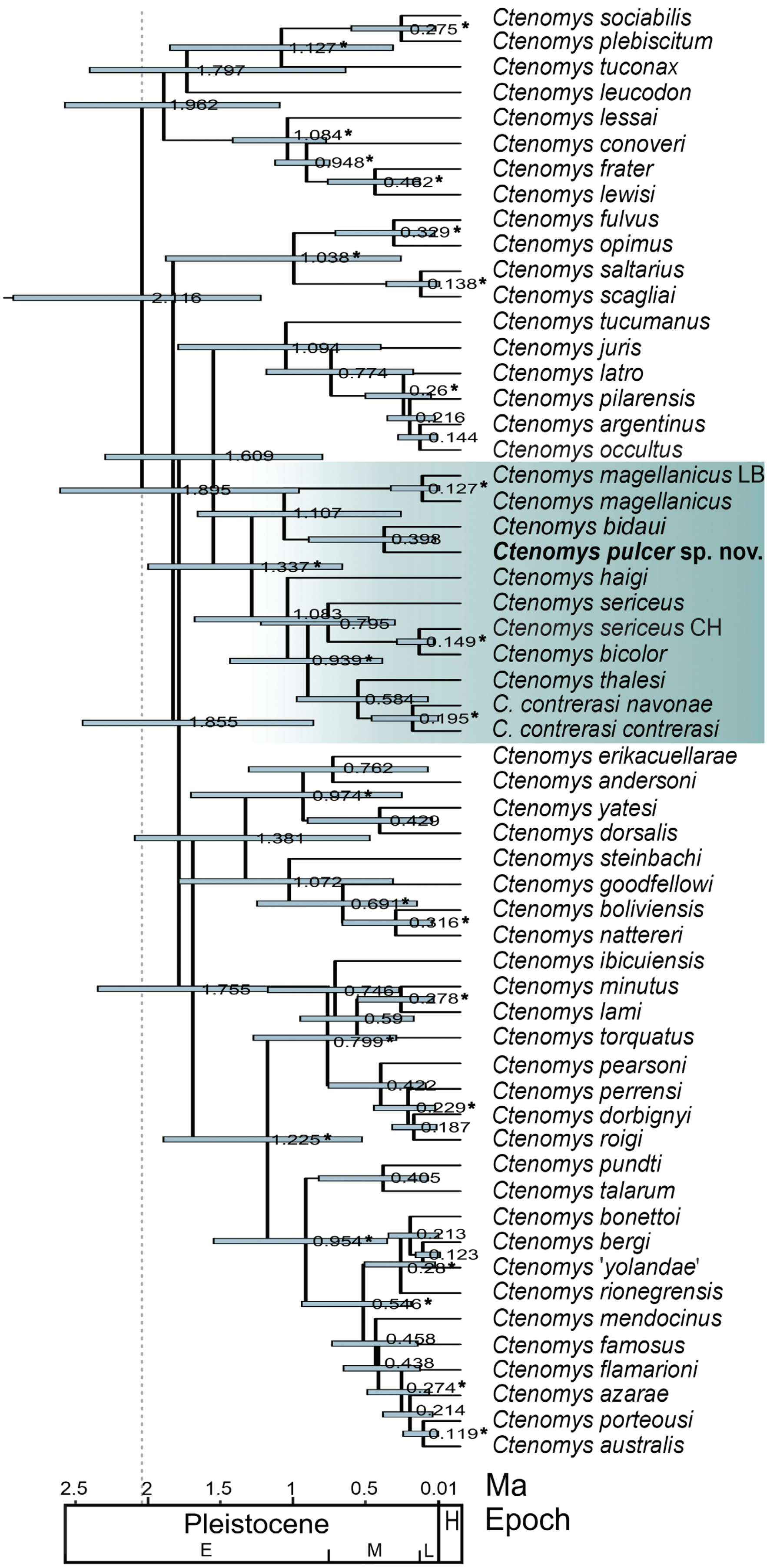

Figure 12. Calibrated phylogenetic tree of *Ctenomys* species obtained through Bayesian analysis of a set of 11 genes (see text). Node numbers and bars indicate estimated ages (in Myr) and 95% credibility intervals, respectively, for each node. The asterisk indicates support of ≥ 0.99 posterior probability. Abbreviations: **CH** Chile Chico (Aysén, Chile); **H** Holocene; **LB** Lago Buenos Aires County (Santa Cruz, Argentina).

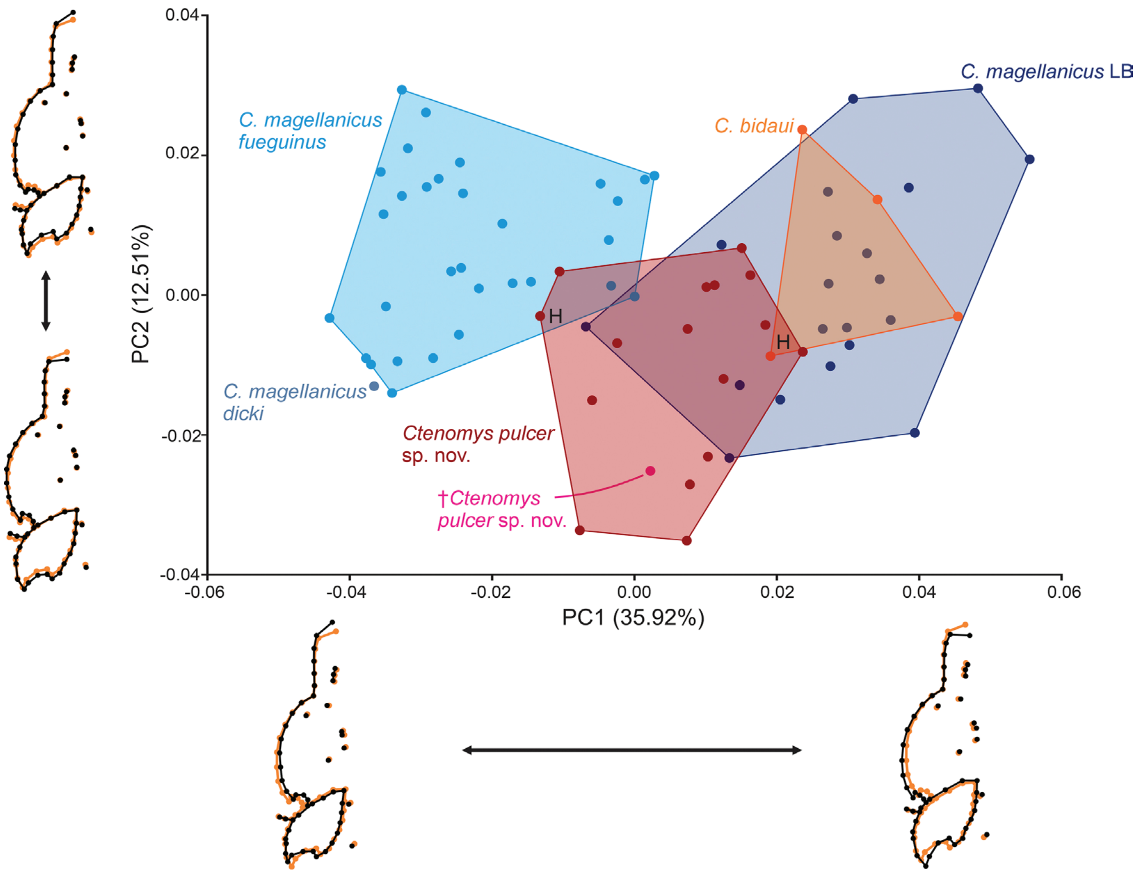

Figure 13. Ordination of *Ctenomys pulcer* **sp. nov.**, *Ctenomys bidaui*, *Ctenomys magellanicus fueguinus*, *Ctenomys magellanicus dicki* and *Ctenomys magellanicus* LB in the morphospace defined by the first two principal components of the aligned Procrustes coordinates (APC) of ventral view of cranial shape variation. Shape changes associated with positive and negative values of both axes are shown as wireframes: black dots and lines indicate shape changes with respect to the mean configuration indicated with orange dots and lines. Abbreviations: H, holotype; LB, Lago Buenos Aires County (Santa Cruz, Argentina).

are grouped by the following shared character-states: premaxillary margins at the level of the root of premaxillary septum converging dorsomedially (character-state 17–2); facial portion of the lacrimal scarcely developed (character-state 21–1) and not protruding with respect to the frontal (character-state 22–1); orbital portion of the lacrimal interrupted by the frontal bone (character-state 23–1); dorsal part of the nasolacrimal canal very short (character-state 24–1), rather anteriorly oriented (character-state 25–1), and with its posterior margin scarcely differentiated or absent (character-state 26–1); laterodorsal portion of the alveolar sheath of M1 covered by the maxillary plate that delimits the sphenopalatine fissure (character-state 28–1). These synapomorphies for living *C. pulcer* –†*C. pulcer* are shown in the character mapping on the strict consensus phylogeny of Fig. S2.

Bayesian analysis

The topology resulting from the node-dating Bayesian analysis of the molecular dataset was essentially similar to the one obtained in the maximum parsimony analysis on the combined matrix. Again, eight of the recovered clades are consistent with the recognized species groups, while the relationships among these clades are essentially the same as those in the consensus parsimony tree (Fig. 12). With the exception of the *boliviensis* and *tucumanus* groups, the rest of the clades corresponding to the species groups are well supported (PP $\geq$ 0.99). The unexpected placement of *Ctenomys bicolor* in the *magellanicus* species group is probably a result of the heterogeneous representation of genes across the sample. The initial divergence within the crown group is estimated to have occurred during the early Pleistocene, near 2 Ma. The divergences that gave rise to the species groups and the initial splits within each of them are estimated to have occurred mainly during the early Pleistocene, except for the younger clades *talarum* and *mendocinus* which would have begun to diversify during the middle Pleistocene. The divergence between *Ctenomys pulcer* and *C. bidaui*, within the *magellanicus* clade, is estimated to have occurred in the middle Pleistocene, around 0.4 Ma.

Geometric morphometrics

The morphospace of ventral cranium variation, delimited by the first two principal components (PC1 and PC2) of the aligned Procrustes coordinates, explained 48% of the total shape variation (PC1 35.92%, PC2 12.51%; Fig. 13). Living *Ctenomys pulcer* and fossil †*Ctenomys pulcer* were distributed at intermediate scores along PC1 (i.e., from low negative scores to low positive scores), separated from *C. bidaui*; this portion of the morphospace was characterized by a relatively elongated rostrum, wider incisive foramina and palatal bridge, medially shifted zygomatic arch and glenoid fossa, larger postglenoid articular region, anterolaterally less inflated auditory bulla, and paraoccipital process more laterally expanded on the mastoid bulla. *Ctenomys bidaui* was located on higher positive scores of the PC1 and showed opposite characteristics for these morphological traits. Notably, *C. magellanicus* from Tierra del Fuego Island and *C. magellanicus* from Lago Buenos Aires were clearly separated along this first axis. Instead, *C. bidaui* was completely overlapping with *C. magellanicus* LB while *C. pulcer* was partially overlapping with *C. magellanicus* LB and *C. magellanicus fueguinus* (see Discussion). PC2 was an axis of intrapopulational variation. *Ctenomys* specimens with longer diastema and slightly smaller auditory bulla were located on positive values of this axis, while those with opposite traits were distributed along negative scores.

The morphospace of lateral cranial variation defined by the first two principal components of the PCA on the aligned Procrustes coordinates summarized more than 37% of the total shape variation (PC1 23.08%, PC2 14.59%; Fig. S3). This morphospace did not segregate species or subspecies but was largely a space of intrapopulational variation. Specimens with shorter rostrum, more anterior zygoma, more vaulted braincase and inflated auditory bulla were distributed on negative scores of PC1 whereas those with opposite traits presented positive values along this axis. On PC2, specimens with slightly more vaulted braincase and larger auditory bulla were distributed along negative scores (Fig. S3).

Discussion

As mentioned in the Introduction, the accelerated diversification process of *Ctenomys* (Upham et al. 2021) has resulted in evolving lineages at different stages of differentiation. This, often associated with a remarkable morphological homogeneity even among long-differentiated species (D'Elía et al. 2021), makes species boundaries difficult to define. Here we follow the unified species criterion proposed by de Queiroz (2005), according to which a species is a metapopulation lineage evolving separately from other such lineages. Under this concept, phenetic distinction, reproductive isolation, or ecological divergence are secondary, contingent rather than necessary properties of species as biological entities. However, although this criterion eliminates incompatibilities between alternative species concepts, it does not solve the operational problem of delimiting species. We agree with D'Elía et al. (2021) on the need to increase sampling efforts, as well as to conduct simultaneous analyses from different sources of evidence to assess the distinctiveness of potential species. If such an integrative taxonomic approach is desirable to achieve robust systematic results for any clade, in the case of *Ctenomys* this practice is a requisite starting point for interpreting the taxonomic status of populations under study (see Freitas et al. 2012; Gardner et al. 2014; Teta and D'Elía 2020; D'Elía et al. 2021). The history of the knowledge of *Ctenomys pulcer* is an example of the need for analyses that bring together different sources of evidence. This species was alternatively interpreted as part of the variation of *C. talarum* in cytogenetic (Massarini et al. 1995), paleontological (Massoia 1988; Pardiñas 2001), and phylogeographic studies (Mora et al. 2007), or considered an independent lineage (Morgan and Verzi 2006, 2011) erroneously assigned to the *mendocinus* group according to morphological information in combined phylogenies (De Santi et al. 2018; De Santi 2022). The results obtained here by assembling morphological evidence and molecular markers indicate that it belongs to the Patagonian *magellanicus* species group, within which it is sister to the recently described *C. bidaui* (Teta and D´Elía 2020). Within the variation boundaries of its clade, and compared with *C. bidaui*, this lineage shows a genetic (p-distance) and morphological divergence that allow it to be reliably accepted as an independent species. In this regard, its separation from *C. bidaui* along the first axis of the morphospace of ventral cranial shape by the lesser anterolateral inflation of the auditory bulla and medial displacement of the posterior zygomatic arch and postglenoid fossa is eloquent. Notably, these changes appear to be concerted in this case. However, in the first axis of a ventral cranial morphospace generated through the same landmark configuration for a comprehensive sample of 63 living species, De Santi (2022) found that such changes in the auditory bulla and zygomatic arch follow opposite directions (i.e., an anterolaterally less inflated bulla is accompanied by a laterally displaced zygomatic arch) and are largely size-mediated (cf. De Santi 2022: figs 64 and 66). Thus, it is not to be expected that this difference detected between *Ctenomys pulcer* and *C. bidaui* could represent simple size-mediated variation. The separation of these lineages in the ventral cranial shape analysis but not in the analysis of lateral shape is consistent with recent results showing that in *Ctenomys* the ventral cranial norm bears greater phylogenetic information than the lateral norm (De Santi 2022). In any case, the usefulness of geometric morphometrics of skull or mandible for species delimitation in *Ctenomys* is limited (see De Santi et al. 2021; De Santi 2022) and must be accompanied by qualitative morphological information. In this study, the variation in qualitative morphology detected between *Ctenomys pulcer* and *C. bidaui* is also meaningful. This includes shared apomorphies of the extant and fossil populations of *C. pulcer* that are only found in these samples or are recorded as homoplasies outside the *magellanicus* clade (Fig. S2). Notably, the degree of development of the ventral spine of the styliform process of the auditory bulla in the living population of *C. pulcer* was found to be unique within the comprehensive sample analyzed, suggesting that expression of this character-state was fixed within the last ca. 8,900 years (see below and Fig. S2). Recently, Tammone et al. (2022) described new populations of *C. bidaui* from central Argentina as part of a geographically comprehensive study of *Ctenomys*. These populations remain to be included in phylogenetic analyses of combined evidence to test their relationships with *C. pulcer*.

As mentioned, *C. pulcer* occurs in parapatry with two other species belonging to two different species groups of *Ctenomys*, namely *Ctenomys australis* (*mendocinus* group) and *Ctenomys talarum* (*talarum* group). According to the fossil record from Monte Hermoso I, this peculiar pattern of parapatry already occurred at least ca. 8,900 yrs ago (Pardiñas 2001; our results). The 20 km shore-perpendicular transect conducted in the area showed differential occupation of the environments, with *C. australis* occupying the living dunes of the coast, *C. pulcer* the fixed and semifixed dunes, and *C. talarum* the more humified inland soils, where it was restricted mostly to the edges of cultivated lands. This segregation of habitats is consistent with the greater potential tooth-digging capacity of *C. talarum* relative to the other two species (Morgan et al. 2017; see Vassallo 1998), which allows it to occupy harder soils with higher density of plant roots. This differential habitat use by these three parapatric species offers a unique opportunity for further ecomorphological analyses that should include *C. pulcer* as previously done with *C. talarum* and *C. australis* (Vassallo 1998; Vieytes et al. 2007).

According to the Bayesian node-dating analysis, *C. pulcer* and *C. bidaui* diverged from a common ancestor at around 0.4 Ma. This divergence is among the oldest estimated here for pairs of sister species of *Ctenomys* (see Fig. 12). The process underlying the divergence and differentiation of *C. pulcer* remain to be analyzed (see a review for *Ctenomys* in Freitas 2021). Beyond this, and similarly to what was proposed for the parapatric *C. australis*, the current presence of *C. pulcer* in the coastal region of central Argentina is probably a relict of a wider and continuous distribution of potentially suitable environments, especially sandy friable soils (see Massarini and Freitas 2005; Freitas 2021). This is to be expected, especially since the weak specialization of *C. pulcer* for scratch- and tooth-digging (Morgan et al. 2017; De Santi 2022) could represent a constraint for the occupation of more compact soils with higher plant root density. In this sense, two available paleoenvironmental models, not mutually exclusive, support the development of large extensions of eolian sand and loess in the Chaco-Pampean plains during the late Pleistocene-middle Holocene. During this interval, dry and cool climate episodes occurred in the Chaco-Pampa plain due to extensive glaciations in Andean and peri-Andean areas. Different, non-synchronous eolian sand and loess deposits through the Chaco-Pampa plains attest to these episodes (Iriondo and García 1993; Szelagowski et al. 2004; Giai et al. 2008; Isla et al. 2010; Kruck et al. 2011). This sedimentological evidence suggests that cold and dry environmental conditions similar to those of current Patagonia extended over 750 km northeastward than today during the late Pleistocene–early Holocene (Tonni et al. 1999). Secondly, a palaeogeographical model of the evolution of the Patagonian and Pampean coasts provided by Ponce et al. (2011) shows that during the Last Glacial Maximum (ca. 24,000 calibrated years BP), sea level was approximately 120–140 m below present values. Thus, a very large portion of the South American continental shelf was exposed, generating a huge coastal plain along the Pampas and Patagonia that extended to the central portion of the Brazilian Atlantic coast. According to sedimentological analyses, the dominant sediments on this platform are sands. Both lines of evidence could explain the current distribution of *C. pulcer* in the present-day coastal margin of the Pampean phyto- and biogeographic province in central Argentina (Cabrera 1971; Cabrera and Willink 1973; Morrone 2014), stemming from a putative Patagonian ancestor (see Fig. 2; Tammone et al. 2022).

The current area of distribution of *C. pulcer* is occupied by coastal dunes and associated communities that represent particularly fragile natural systems (Monserrat and Codignotto 2013; Celsi et al. 2016). The area is currently subjected to profound disturbances caused by diverse anthropic actions, including establishment and expansion of urban centers, exotic forest plantings, building of new roads and coastal defense structures, and intense circulation of off-road vehicles. These processes have caused significant reduction of natural habitats, as well as their fragmentation and modification, with the consequent negative impact on biodiversity (Dadon and Matteucci 2002; Celsi 2016). In addition to the more obvious ecological problems such as changes in the native plant communities that constitute food resources for *Ctenomys* and other herbivorous species (Monserrat et al. 2012), other environmental disturbances may also be relevant for subterranean species. In particular, alterations of the physico-chemical characteristics of the soil and of soil-forming processes such as the eolic transport and deposit of sand (Marcomini et al. 2011) brought about by human action, may directly affect the burrowing ability of the relatively non-specialized *C. pulcer*.

The human footprints overlying the levels with *C. pulcer* at the Monte Hermoso I site, together with the older ones at the neighboring Pehuen-Co paleoichnological site (Bayón et al. 2011) attest that for at least 8,900 years *C. pulcer* has coexisted with the human species in the Pampean area. The paleontological and archaeological sites of Monte Hermoso and Pehuen-Co areas have been part of a protected natural reserve since 2006, but this natural reserve does not include the habitat of *C. pulcer* (Celsi et al. 2016). New protection strategies are needed to ensure that *C. pulcer*, its natural environments, vegetation and accompanying fauna continue to coexist with us. In this sense, a first initiative should include the monitoring of the natural reserve "Reserva Natural de la Defensa Baterías-Charles Darwin" located at the west of Monte Hermoso, which partially includes environments such as those occupied by *C. pulcer* (Celsi et al. 2016).

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Competing interests

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Supplementary Material 1

Table S1

Authors: Verzi DH, De Santi NA, Olivares AI, Morgan CC, Basso NG, Brook F (2023)
Data type: .pdf
Explanation note: List of taxa and specimens examined.

Link: https://doi.org/10.3897/vz.73.e96656.suppl1

Supplementary Material 2

Table S2

Authors: Verzi DH, De Santi NA, Olivares AI, Morgan CC, Basso NG, Brook F (2023)
Data type: .pdf
Explanation note: List of Genbank accession numbers for sequences used in this study.

Link: https://doi.org/10.3897/vz.73.e96656.suppl2

Supplementary Material 3

Table S3

Authors: Verzi DH, De Santi NA, Olivares AI, Morgan CC, Basso NG, Brook F (2023)
Data type: .pdf
Explanation note: Description of characters used in the phylogenetic analysis.

Link: https://doi.org/10.3897/vz.73.e96656.suppl3

Supplementary Material 4

Table S4

Authors: Verzi DH, De Santi NA, Olivares AI, Morgan CC, Basso NG, Brook F (2023)
Data type: .pdf
Explanation note: Fossil constraints.

Link: https://doi.org/10.3897/vz.73.e96656.suppl4

Supplementary Material 5

Table S5

Authors: Verzi DH, De Santi NA, Olivares AI, Morgan CC, Basso NG, Brook F (2023)
Data type: .pdf
Explanation note: Morphometric analysis. Description of cranial landmarks and semilandmarks.

Link: https://doi.org/10.3897/vz.73.e96656.suppl5

Supplementary Material 6

Figure S1

Authors: Verzi DH, De Santi NA, Olivares AI, Morgan CC, Basso NG, Brook F (2023)
Data type: .pdf
Explanation note: Simple-asymmetric sperm of *Ctenomys pulcer* **sp. nov.** (MLP-Mz 2538, Sauce Grande lagoon, Monte Hermoso, Argentina). Photograph by Martino NS.

Link: https://doi.org/10.3897/vz.73.e96656.suppl6

Supplementary Material 7

Figure S2

Authors: Verzi DH, De Santi NA, Olivares AI, Morgan CC, Basso NG, Brook F (2023)
Data type: .pdf
Explanation note: Mapping of selected characters.

Link: https://doi.org/10.3897/vz.73.e96656.suppl7

Supplementary Material 8

Figure S3

Authors: Verzi DH, De Santi NA, Olivares AI, Morgan CC, Basso NG, Brook F (2023)
Data type: .pdf
Explanation note: Ordination of *Ctenomys pulcer* **sp. nov.**, *Ctenomys bidaui*, *Ctenomys magellanicus fueguinus*, and *Ctenomys magellanicus* LB in the morphospace defined by the first two principal components of the aligned Procrustes coordinates (APC) of lateral view of cranial shape variation.

Link: https://doi.org/10.3897/vz.73.e96656.suppl8