

SUPPLEMENTARY MATERIALS

Table S1

Specimens examined: the locality of collection (country, region/ department/ province, district, locality).

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Anotomys leander ($n = 7$): Ecuador, Napo, 6.2 km of Papallacta by road (QCAZ 6256); Quijos, 6.9 km of Papallacta by road (AMNH 244605-24407); Pichincha, Quito Mount Pichincha (AMNH 66201, 66202); Colombia, Municipio de Cajamarca (CTUA 2556).

Chibchanomys trichotis ($n = 3$): Colombia, Cundinamarca, Pasca, Vereda Chisaca, Lake Rebosadero (ICN 10152); Santander, Finca El Rasgón (CTUA s/n), (MHN-UIS s/n examined through digital photographs); Venezuela, Táchira, Buena Vista, 41 km SW of San Cristóbal (USNM 442606).

Daptomys ferreirai ($n = 2$): Brazil, Pará, Pacajá (X1M 27); Rondônia, Pacaás Novos (MTR 25579). Both examined through digital photographs.

Daptomys mussoi ($n = 1$): Colombia, Santander, Municipio Chigatá, Vereda San Carlos (ICN 21295).

Daptomys oyapocki ($n = 1$): Guyana Francesa, Cayena, Sinnamary, Paracou (AMNH 267597).

Daptomys peruviansis ($n = 10$): Ecuador, Pastaza, Comunidad Kurintza, Río Villano (QCAZ 10352); Zamora-Chinchipe, Los Encuentros, El Cóndor, Luchin Gold (MECN 6629); Orellana, Onkone Gare, 38 km S of Pompeya Sur (ROM 118768, 118812, both examined through digital photographs); Perú, Madre de Dios, Manu, Manu, Río Manu, Parque Nacional del Manu, Puesto de Vigilancia de Pakitza (MUSM 9214); Ucayali,

Coronel Portillo, Iparía, río Shesha (MUSA 12675); Cusco; Paucartambo; Villa Carmen (MUSA 19658); Loreto, Distrito de San Juan Bautista, Llanchara (MUSM 45735, 44974, 44975)

Daptomys venezuelae ($n = 3$): Guyana, Cuyuni, Mazaruni Región: Kartabo Point AMNH 142818; Venezuela, Amazonas, cerro Duida (USNM 406123); Sucre, Neverí (AMNH 69908).

Daptomys spp. ($n = 3$) Bolivia, La Paz, 0.5 km east Saynami, río Zongo (TLY 1519, examined through digital photographs); Perú, San Martín, Rioja, Santa Elena.

Ichthyomys hydrobates hydrobates ($n = 14$): Colombia, Cauca (ICN 10029); Munchiquito mountain (USNM 294985); Napo, Las Caucheras, Cosanga, Río Aliso (MEPN 5935); Venezuela, Mérida, Libertador (AMNH 24351, 24354-24357, USNM 244610); near Mérida (USNM 123323, 12325, 151288, 172941); Unknown locality (USNM 115315).

Ichthyomys hydrobates soderstomi ($n = 20$): Ecuador, Pichincha, Quito, Guapulo (AMNH 46730-46733, 64624); Pachijal (MEPN 5832-5835); Machangara river (AMNH 46729); Río Blanco, Cacao Cocha (AMNH 244610); Río Blanco, Quinindé (MEPN 5829-5831); Bolívar, Cashca Totoras, Bosque Protector Cashca Totoras (QCAZ 907); Zamora-Chinchipe, 4 km E Sabanilla (USNM 513625); Cotopaxi, San Francisco de Las Pampas, San Francisco de Las Pampas (QCAZ 818); Unknown locality (AMNH 39593, 39594); Chimborazo, Pallatanga, Los Santiagos (MECN 3960).

Ichthyomys orientalis ($n = 3$): Ecuador, Morona-Santiago, río Jurumbo, Nueva Alianza (MECN 4914); Tungurahua, Baños Río Zuñac (MEPN 12672); Zamora-Chinchipe, Sabanilla, 4 Km E (USNM 513625).

Ichthyomys pinei ($n = 2$): Ecuador, Azuay, La Playa, río León, Parroquia and Cantón Nabón (MZUA 234 (Holotype), MECN 5613).

Ichthyomys pittieri ($n = 3$): Venezuela, Aragua, Rancho Grande (USNM 562980); Carabobo, Palmichal, 23 km N. Bejuma (USNM 562981); Unknown locality (USNM 562983).

Ichthyomys stolzmanni ($n = 10$): Perú, Ayacucho, Huamanga, Vinchos, restaurant El Bagrecito (MUSM 25807), Piscigranja Arizona (MUSM 25808, 25809), Jatumpampa (MUSA 16431, 17792, 18919, 18920); Cajamarca, Cajabamba (AMNH 10109); Cusco, Urubamba, Aguas Calientes, Mandorpampa (MUSA 18953, 18954).

Ichthyomys tweedii ($n = 18$): Ecuador, Pichincha, San Miguel de los Bancos: Mindo, Pechahal (AMNH 71382-71392); Imbabura, Paramba (MECN 2156); El Oro, Santas Rosa (MECN 5772); Azuay, Ponce Enríquez, río Tenguel (MEPN 7377, 12229). Panamá, Aguacate, Near Lidice (USNM 460684, 461078, 461094).

Incanomys mayopuma **sp. nov.** ($n = 4$): Perú, Cusco, Urubamba, Aguas Calientes, Wiñaywayna (MUSA 13864, 18964, 18965); Huánuco. Carpish, Cordillera de Carpish, Carretera Central (LSU 14406 examined through digital photographs).

Neusticomys monticolus ($n = 33$): Colombia, Antioquia, Andes, Vereda La Soledad, Finca La Reina (ICN 16526); Ecuador, Bolívar, Guaranda; Sinchig (AMNH 66848); Sinche, Paujchi (AMNH 62920); Páramo de Culebrillas (QCAZ 9866); Pichincha, Quito, Las Máquinas (AMNH 64634-

64639); Pita river canyon (AMNH 64628-64630); San Rafael river (AMNH 64627); Santa Rosa (AMNH 64631-64633, 64646); Santa Rosa, Pita river (AMNH 63376); Río Tandayapa (QCAZ 6446, 6531); Otonga (QCAZ 8688, 8690, 8691, 13066, 15033); San Ignacio (AMNH 64625, 64626); Chimborazo, Chimborazo, Reserva de Producción Faunística (MECN 6366); Carchi, Jesús del Gran Poder, Loma Guagua (QCAZ 9888); Tulcán, Qda. Negra, Bosque la Esperanza, Chilcal (MECN 6305, 6331); Unknown locality (AMNH 69980).

Neusticomys orcesi ($n = 6$): Ecuador, Azuay, Parque Nacional Las Cajas (MEPN 11230); Las Cajas, Lake Luspa (BMNH 82.816 (holotype), 82.815, both examined through digital photographs); Cuenca, Estrellas Cocha (MZU-MA-037); El Oro, Chilla, Cabecera del río Casacay (MECN 6185, 6186)

Neusticomys vossi ($n = 20$): Ecuador, Carchi, Reserva Ecológica El Ángel (QCAZ 12530-12532); Jesús del Gran Poder, Loma Guagua (QCAZ 9888); Imbabura, Zuleta, Hda. San Pedro (QCAZ 11667-11672); Napo, Papallacta, (QCAZ 4145, 6257), Quijos, 1.6 km east of Papallacta (AMNH 244608, 244609); Bermejo, 11 km SE de Baeza por carretera (QCAZ 7830, Holotype); volcán Sumaco (QCAZ 8956); Reserva Ecológica Cuyambe-Coca (QCAZ 927); Morona-Santiago, Morona, Parque Nacional Sangay, sector San Balón (MECN 4332); Napo, Papallacta, bosque administrado por la fundación Terra (QCAZ 4145, 6257);

Neusticomys spp. ($n = 3$): Colombia, Rirasalda, Pereira, Vereda La Suiza, Qda. La Hacienda (ICN 12118); Venezuela, Bolívar, 5.2 km northeast of San Ignacio de Yuruani, on road to toma de Agua (AMNH 257344, 257345);

Rheomys mexicanus ($n = 24$): México, Oaxaca, Juchitan, Unión Hidalgo (AMNH 205313, 205316-205319, 208255-208257); Juchitan, Unión Hidalgo, Chicapa River (AMNH 185015-185018, 189291, 205314, 205315); Miahuatlan, San José Lachiguirí (AMNH 175423, 179970-179972, 182139, 186922, 205320, 205321, 207447).

Rheomys raptor hartmanni ($n = 4$): Costa Rica, Puntarenas, Guacimal river, Lawton Crossing, (USNM 565826); Panamá, Bocas del Toro, First Water or Upper Camp-1975 (USNM 520769); Chiriquí, Cotito Hot Springs (USNM 396585, 396586).

Rheomys raptor raptor ($n = 2$): Panamá, Darién, Mount Pirre, río Limón, Near Head of (USNM 109026, 179029).

Rheomys thomasi chiapensis ($n = 1$): México, Chiapas, Ixhuatan: 1.8 mi west of Ixhuatan (AMNH 204261);

Rheomys thomasi thomasi ($n = 2$): El Salvador, Ahuachapan, La Fincona, El Imposible, San Francisco Menendez (ROM 101294); El Salvador, Huehuet, Chiantha, Laguna Magdalena (JRG s/n).

Rheomys underwoodi ($n = 3$): Costa Rica, Rirasalva, Alturas de Cotón (MNCR s/n). Panamá, Chiriquí, El Volcán, 17 km NNW, Head of río Candela (USNM 516939, 516940).

Table S2. Qualitative morphological characters and character states selected for phylogenetic analysis are described below and the distribution of their states among ichthyomyine species; and matrix of morphological character states of species of Ichthyomyini tribe.

Species \ Character	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	
<i>Anotomys leander</i>	1	1	1	1	0	1	1	0	1	3	0	1	1	1	2	0	2	2	0	0	1	0	0	1	0	0	0	0	0	1	0	0	0	1	0	1	0	0	1
<i>Chibchanomys trichotis</i>	1	1	1	1	0	1	0	0	1	2	1	0	2	1	2	2	2	2	0	0	1	0	1	0	1	0	0	2	2	1	1	0	0	0	0	0	0	0	0
<i>Incanomys new species</i>	1	1	1	1	0	1	0	0	1	2	1	0	2	1	2	2	2	2	0	0	1	0	1	0	1	0	0	2	2	1	1	0	0	0	0	0	0	0	0
<i>Ichthyomys hydrobates</i>	2	1	2	0	1	0	0	0	0	1	1	0	2	1	2	1	2	0	1	1	0	1	3	2	0	1	1	2	2	1	1	0	2	1	0	1	1	0	
<i>Ichthyomys pittieri</i>	2	1	2	0	1	0	0	0	0	1	1	0	2	1	2	1	2	0	1	1	0	1	3	2	0	1	1	2	2	1	1	1	2	1	0	1	1	0	
<i>Ichthyomys stolzmanni</i>	2	1	2	0	1	0	1	1	0	1	1	0	2	1	2	1	2	0	1	1	0	1	3	2	0	1	1	2	2	1	1	0	2	1	0	1	1	0	
<i>Ichthyomys orientalis</i>	2	2	2	0	1	0	0	1	0	1	1	0	2	1	2	1	0	0	1	1	0	1	3	2	0	1	1	2	2	1	1	0	2	1	0	1	1	0	
<i>Ichthyomys tweedii</i>	2	1	2	0	1	0	1	0	0	1	1	0	2	1	2	0	0	0	1	1	0	1	3	2	0	1	1	2	2	1	1	0	2	1	0	1	1	0	
<i>Neusticomys monticolus</i>	0	1	0	1	0	2	0	0	0	0	1	0	0	0	1	0	0	1	0	0	1	0	1	0	0	0	0	2	2	0	2	1	2	0	0	0	0	0	
<i>Neusticomys orcesi</i>	1	1	0	1	0	1	0	0	0	0	1	0	0	0	1	0	1	1	0	0	1	0	1	0	0	0	0	2	2	1	2	1	2	1	0	0	0	0	
<i>Neusticomys vossi</i>	0	1	0	1	0	2	0	0	0	0	1	0	0	0	1	0	0	1	0	0	1	0	1	0	0	0	0	2	2	0	2	1	2	0	0	0	0	0	
<i>Daptomys ferreirai</i>	1	2	0	0	0	1	0	0	0	0	1	0	0	0	1	0	0	0	0	1	0	0	1	0	1	1	0	1	2	0	2	0	1	1	0	0	0	0	
<i>Daptomys mussoi</i>	0	2	0	1	0	1	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	1	0	1	0	0	0	2	2	0	2	0	1	1	0	0	0	0	
<i>Daptomys oyapocki</i>	1	2	0	0	0	1	0	0	0	0	1	0	0	0	1	0	0	0	0	1	0	0	1	0	1	1	0	1	2	0	2	0	1	1	0	0	0	0	
<i>Daptomys peruviansis</i>	1	2	0	0	0	1	0	0	0	0	1	0	0	0	1	0	0	0	0	1	0	0	1	0	1	1	0	1	2	0	2	0	1	1	0	0	0	0	
<i>Daptomys venezuelae</i>	1	2	0	0	0	2	0	0	0	0	1	0	0	0	1	0	0	0	0	1	0	0	1	0	1	1	0	2	1	0	2	0	1	1	0	0	0	0	
<i>Rheomys mexicanus</i>	2	0	1	1	1	1	0	1	1	2	1	2	2	1	2	1	2	1	0	0	1	0	2	2	0	0	0	2	1	0	1	1	0	1	1	1	0	2	
<i>Rheomys raptor</i>	0	0	0	1	0	1	0	0	0	0	1	1	2	0	1	0	0	1	0	0	1	0	2	2	0	0	0	2	1	0	1	0	0	1	0	1	0	2	
<i>Rheomys thomasi</i>	1	0	0	0	0	1	0	1	0	2	1	1	1	1	2	0	2	1	0	0	1	0	2	2	0	0	0	2	1	0	1	0	0	1	0	1	0	2	
<i>Rheomys undewoodi</i>	2	0	1	1	1	0	0	1	1	2	1	2	2	1	2	1	2	1	0	0	1	0	2	2	0	0	0	2	1	0	0	0	0	1	0	1	0	2	
<i>Sigmodon</i>	2	1	0	1	1	2	1	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	1	0	1	0	2	2	0	0	0	0	0	0	0	0	0	
<i>Nectomys</i>	2	2	0	1	1	1	0	0	0	0	0	0	2	0	0	0	0	0	0	0	1	1	0	1	2	0	1	0	2	2	0	0	0	0	0	0	0	0	
<i>Holochilus</i>	2	2	0	0	0	1	0	0	0	0	0	0	2	0	0	0	0	0	0	0	1	1	0	1	2	0	1	0	2	2	0	0	0	0	0	0	0	0	

1. Body mass — 3 states: 0 = Small mice (22 – 30 g); 1 = Medium mice (35 – 65 g); 2 = Large rat (80 – 158 g).

We discriminate three states unlike Salazar-Bravo et al. (2023) which only uses two. All measurements were taken from labels, or data provided by the authors. Adult ichthyomyines varies in body mass (22 to 158 g in adults). Small animals with less than 35 g in *R. raptor* (includes: *hartmanni*), *Neusticomys vossi*, *N. monticolus* and *D. mussoi*; medium weigh 35 to 65 g, in *A. leander*, *Chibchanomys*, *Incanomys*, *Daptomys*, and *R. thomasi*; and large animals with weights above 70 g, in *Ichthyomys* spp. *R. underwoodi*, and *R. mexicanus*.

2. Biogeographical distribution — 3 states: 0 = Central America; 1 = Andean and around the Andes; 2 = Low lands to east of the Andes

Ichthyomyini genera have a differential geographical distribution. All species of *Rheomys* lives only in Central America; *A. leander*, *Chibchanomys*, *Incanomys*, *Neusticomys*. and *Ichthyomys* spp. are Andean species, but some species inhabits in the western slopes in Ecuador and *I. tweedii* has a population in Panama and Costa Rica; and *Daptomys* species are present on Amazon lowlands and the last foothills of the eastern Andes below 1500 m to near sea level, from Venezuela to Bolivia.

3. Body design — 3 states: 0 = Mouse-like; 1 = Transitional; 2 = Fusiform and elongate

Aquatic life-style required various aquatic adaptations and adjustments in organization of these animals (Kukenthal 1891, Fish 1996, Fish 2016, Houssaye and Fish 2016). The better indicator of swimming performance is the possession of a fusiform and elongate body (Kukenthal 1891, Fish 1996). Body design of ichthyomyines showed three morphologies, the least modified keeps the shape of a mouse, in *Neusticomys*, *Daptomys*, and *R. raptor* with developed ears and hind foot least specialized for aquatic life (Anthony 1921); intermediate design species with medium-sized with small arms, very small ears and large and broad hindfeet, includes to *A. leander*, *C. trichotis*, *Incanomys*; *R. thomasi* and *R. underwoodi*; large, elongated and fusiform in *Ichthyomys* species.

4. Body pelage — 2 states: 0 = Pelage glossy and grizzled-brownish; 1 = Pelage dull and gray-black

In this Character (figure 2) we follow the character 1 of Voss (1988). Glossy and grizzled-brownish found in *Ichthyomys* and *Daptomys*, *R. raptor*, *R. underwoodi* and *R. mexicanus*, dull and gray black with fine scattered guard hairs fund in *Chibchanomys*, *Neusticomys*, *A. leander* and *R. thomasi*.

5. Guard hairs — 2 states: 0 = Comparatively few or not abundant or inconspicuous; 1 = Long and abundant

In relation to the amount of guard hairs, two conditions have been defined (Anthony 1929, Salazar-Bravo et al. 2023), one with inconspicuous guard-hairs with few fine scattered guard hairs that are present in *A. leander*, *Chibchanomys*, *Incanomys*, *R. raptor*, *R. thomasi* and *Neusticomys*. Other character state with abundant guard-hairs interspersed with wool hairs found in *Ichthyomys*, *R. mexicanus* and *R. underwoodi*.

6. Ventral countershading — 3 states: 0 = Strong countershading; 1 = Weak countershading; 2 = Not countershading

We follow to Voss (1988), Weksler (2006), and Salazar-Bravo et al. (2023). Dorsal and ventral colors sharply delimited with a marked border, dorsum darker than the pale ventral surface in *Ichthyomys* and *R. mexicanus*. Dorsal and ventral colors subtly delimited, dorsum slightly darker than ventral surface, resulting in weak countershading, in *A. leander*, Ecuadorian form of *N. orcesi*, *C. trichotis*, *R. undewoodi*, part of *Daptomys*. Limits of dorsal and ventral colors indistinct, ventral surface dark, not countershading in *Neusticomys*, *Incanomys*, and *Daptomys venezuelae*.

7. Pale spots on auricular region — 2 states: 0 = Absent; 1 = Present

Ichthyomyini rodents present some white or yellowish spots on the auricular region. Salazar-Bravo et al. (2023), and we include species that have light hairs on their ears. White in *Anotomys*, less conspicuous in *I. stolzmanni* and *I. orientalis*; absent in *Chibchanomys*, *Daptomys*, *Incanomys*, *Neusticomys*, *Rheomys* and the rest of *Ichthyomys*.

8. Tail color pattern — 2 states: 0 = Unicolored; 1 = Bicolored

This character distinguishes the color pattern of tail of Ichthyomyines, we follow the Character 3 of Voss (1988). Most species have a single-colored tail although it may be slightly paler at the bottom, there is not a noticeable difference, but some species have a bicolor pattern, with the dorsal part darker and noticeable pale or whitish in ventral part, we find it in *I. stolzmanni*, *R. underwoodi* and *R. mexicanus*. Salazar-Bravo et al. (2023) use a third state, particolored, according to our observations it is a variable state in some species.

9. Naso-labial sulcus or philtrum — 3 states: 0 = Naked; 1 = Partial; 2 = Hairy

This character is a modification of Character 4 of Voss (1988), also used by Jenkins and Barnett (1997), and Salazar-Bravo et al. (2023) these authors used “present” to describe a hairless state or naked in *Ichthyomys*, *Daptomys*, *Incanomys*, *Neusticomys*, *R. thomasi* and *R. raptor*; and “absent” to describe a state covered with hairs in *A. leander*, *C. trichotis*, *R. mexicanus* and *R. underwoodi*. In this work, we find an intermediate state describing a filter that can be partially covered with hairs towards the rhinarium and naked towards the lips, which we call "partial", which would be equivalent to "indistinct" (Voss 1988, p. 324).

10. Pinna — 4 states: 0 = Complete; 1 = Reduced; 2 = Vestigial; 3 = Absent

We follow to Anthony (1929), Ellerman (1941), Voss (1988), and Salazar-Bravo et al. 2023, but we include a character state. Complete external ear when it is showing a structural pattern similar to other sigmodontine rodents and is visible above the fur of the head, is found in *Neusticomys*, *Daptomys*, *R. thomasi* and *R. raptor*. Reduced pinna, as a structure with a squamiform shape and visible above the fur of the head, is found in *Ichthyomys*. Vestigial when lacking to the functional ear conch, very reduced pinna and concealed into the fur, it is found in *C. trichotis*, *Incanomys*, *R. underwoodi* and *R. mexicanus*. Absent pinna is found in *A. leander*.

11. Superciliary vibrissae — 2 states: 0 = Present; 1 = Absent

We follow the character 6 of Voss (1988), also used by Salazar-Bravo et al. (2023). Superciliary vibrissae are absent in all Ichthyomyini with exception to *A. leander*.

12. Number of plantar pads of manus — 3 states: 0 = Five pads; 1 = Four pads; 2 = Three pads

This character was judged by the number of plantar pads on manus (Ellerman 1941, Voss 1988) also used by Salazar-Bravo et al. (2023). Five pads with hypothenar pad separate, not fused with the third interdigital, is found in *Chibchanomys*, *Ichthyomys* and *Neusticomys*. Four pads with hypothenar and third interdigital pads fused, is found in *A. leander*, *R. thomasi* and *R. raptor*. Three pads, with the hypothenar and thenar pads fused with adjacent third and first interdigital pads, respectively, is found in *R. mexicanus* and *R. underwoodi*.

13. Metatarsal configuration — 3 states: 0 = $III \geq IV > II \gg V > I$; 1 = $IV > III > V > II > I$; 2 = $IV > III > II \geq V > I$

We follow to Starrett & Fisler (1970) and Voss (1988) with some modifications, in addition we define a third state. $III \geq IV > II \gg V > I$, found in *Neusticomys*. $IV > III > V > II > I$ is found in *A. leander* and *R. thomasi*. $IV > III > II \geq V > I$, is found in *Incanomys*, *C. trichotis*, *Ichthyomys*, *R. underwoodi*, *R. raptor* and *R. mexicanus*.

14. Fringing hairs on pes — 2 states: 0 = Weakly developed; 1 = Well-developed

This trait distinguishes the development degree of fringed hairs along of the border of pes and digits in two states (Anthony 1929, Ellerman 1941, Voss 1988, Salazar-Bravo et al. 2023). Weakly developed (less than 2 mm), is found in *Neusticomys*, and *R. raptor*. Well-developed (longer than 2.5 mm) in *A. leander*, *C. trichotis*, *Incanomys*, *Ichthyomys*, *R. mexicanus* and *R. underwoodi*.

15. Hindfoot width — 3 states: 0 = Cursorial; 1 = Narrow; 2 = Broad

This trait described the degree of increase of the propulsive surface of hindfoot from swimming (Anthony 1929). Hindfeet narrow, like the cursorial type or most wide that are present in *Neusticomys*, *R. thomasi* and *R. raptor*. Hindfeet very wide adapted for swimming, in *A. leander*, *C. trichotis*, *Ichthyomys*, *Incanomys*, *R. mexicanus* and *R. underwoodi*.

16. Tail shape — 3 states: 0 = Cylindrical and thin tail; 1 = Tapering tail; 2 = Compressed tail

We modify the proposal of two-character states of Salazar-Bravo et al. (2023). We distinguish three features in relation to appearance of tail. Ancestral tails are approximately cylindrical for the most part, is found in *Neusticomys*, *Daptomys* and *I. pittieri*. Tapering tail describes a tail thickened at proximal part and thin in the distal portion, this state is found in *R. mexicanus*, *R. underwoodi*, *I. hydrobates*, *I. stolzmanni* and *I. tweedii*. The third state describes a lateral flattening of the tail, in *A. leander*, *C. trichotis* and *Incanomys*.

17. Comparison between dorsal and ventral hairs of tail — 3 states: 0 = Equal or sub equal; 1 = Slightly longer; 2 = Longer

Underside with hair developed forming slight keel (Howell 1930, Voss 1988) an adaptation to swimming. Ventral hairs of equal size or slightly longer than dorsal or lateral hairs, is found in *I. tweedii*, *A. leander*, *Neusticomys*, *Daptomys*, *R. thomasi* and *R. raptor*; the hairs on the underside of the tail are slightly lengthened, but not forming a slight keel, is found in *N. orcesi*; the hairs on the underside of the tail are slightly lengthened

and are dense, forming a slight keel, found in *I. hydrobates*, *I. stolzmanni*, *I. pittieri*, *R. underwoodi*, *R. mexicanus*, *C. trichotis* and *Incanomys*.

18. Braincase form — 3 states: 0 = Non-inflated; 1 = Rounded; 2 = Globular

This character distinguishes the comparative form of braincase in adult stage (Anthony 1929, Salazar-Bravo et al. 2023). Not inflated skulls are found in *Ichthyomys*, and *Daptomys*; rounded or lightly inflated found in *N. orcesi*, *Rheomys* and *N. (Neusticomys)*; globular or inflated in *A. leander*, *C. trichotis*, and *Incanomys*.

19. Nasal bones — 2 states: 0 = Long; 1 = Short

The extension of the nasals was evaluated considering if their anterior border conceals or not the incisors and the nasal orifice in dorsal view (Ellerman 1941, Voss 1988, Salazar-Bravo et al. 2023). Long state is produced when anterior border of the nasal conceals the incisors and nasal orifice in dorsal view, condition present in most Ichthyomyini species; short state, when anterior border of the nasal to expose the incisors and nasal orifice in dorsal view, found in *Ichthyomys*.

20. Rostrum — 2 states: 0 = Slender; 1 = Heavy

This character distinguishes the comparative width of the rostrum (Anthony 1929, Salazar-Bravo et al. 2023). Rostrum slender in *A. leander*, *Chibchanomys*, *Rheomys* and *Neusticomys*; rostrum heavy in *Daptomys* and *Ichthyomys*.

21. Development of occipital condyles — 2 states: 0 = Developed and visible on dorsal view; 1 = Tiny developed or non-visible on dorsal view

We follow to Salazar-Bravo et al. (2023). Occipital condyles have a different degree of development and exposure in dorsal view. Visible on dorsal view in *Ichthyomys* and *Daptomys*. Tiny developed or non-visible on dorsal view condition in *Neusticomys*, *Rheomys*, *A. leander* and *Chibchanomys*.

22. Supraorbital foramina — 2 states: 0 = Lateral; 1 = Dorsal

This character distinguishes those supraorbital foramina position in adult stage (Voss 1988). Supraorbital foramina open laterally within orbits in *A. leander*, *Neusticomys*, *Rheomys* and *Chibchanomys*; and supraorbital foramina open dorsally displayed in *Ichthyomys*.

23. Interorbital constriction form — 4 states: 0 = Hourglass; 1 = Large interorbital; 2 = Short interorbital; 3 = Constrained

Interorbital region varies in function of the form and orientation of their borders, we follow to Weksler and Percequillo (2011) and Salazar-Bravo et al. (2023). Interorbital region typical hourglass form with semicircular borders or symmetrically concave is found in *A. leander*. Interorbital comparatively large, borders parallels or sub-parallel in *Chibchanomys*, *Neusticomys*. Interorbital short and borders parallels or lightly constrained

is found in *Rheomys*. And very constrained posteriorly in adult specimens, displayed in *Ichthyomys*.

24. Carotid arterial supply — 3 states: 0 = Pattern 1; 1 = Pattern 2; 2 = Pattern 3

Three patterns of carotid circulation were defined by Voss (1988) from ichthyomyine species in his Character 12. Pattern 1 is found in *Chibchanomys*, *Neusticomys*; pattern 2 is found in *A. leander*; and Pattern 3 (derived from state 2) found in *Ichthyomys* and *Rheomys*.

25. Orbicular apophysis of malleus — 2 states: 0 = Present; 1 = Absent

This character describes the presence of the orbicular apophysis of malleus defined by Voss (1988) in his character 12 and followed by Salazar-Bravo et al. (2023). A distinguishable orbicular apophysis of the malleus is present in most ichthyomyines except in *C. trichotis*, *N. orcesi*, *D. ferreirai*, *D. oyapocki*, *D. peruviansis* and *D. venezuelae*.

26. Incisors — 2 states: 0 = Delicate; 1 = Robust

This character distinguishes the degree of development of maxillary incisors in Ichthyomyine rodents (Voss 1988, Salazar-Bravo et al. 2023). Delicate incisors in *Anotomys*, *Chibchanomys*, *Neusticomys* and *Rheomys*; and heavy incisors in *Ichthyomys* and *Daptomys*.

27. Shape of cutting edge of superior incisors as inverted “v” — 2 states: 0 = Shallow; 1 = Deep

In all Ichthyomyinos, the cutting edge of the incisors in the anterior view is presented in the form of an inverted "v" as a possible adaptation to grab slippery preys. But, in *Ichthyomys* the angle formed is deep. In all other species, the cutting edge is shallow.

28. Anteromedian flexus on M1 — 3 states: 0 = Present; 1 = Shallow; 2 = Absent

Salazar-Bravo et al. (2023) defined two character states, to which we add an intermediate one anteromedian flexus, is present and developed in *Anotomys*. shallow anteromedian flexus is found in *D. ferreirai*, *D. oyapocki* and *D. peruanus*, and absent in *Neusticomys*, *D. venezuelae*, *Ichthyomys*, *Rheomys* and *Chibchanomys*.

29. Anteromedian flexid on m1 — 3 states: 0 = Present; 1 = Shallow; 2) = Absent

The anteromedian flexid is present and very developed in *A. leander*; shallow is found in *N. venezuelae*, *Rheomys*; and absent in *Ichthyomys*, *Neusticomys*, *D. peruviansis*, *D. oyapocki*, and *Chibchanomys*.

30. Molars comparative development — 2 states: 0 = Normal; 1 = Reduced

This character distinguishes the relative development of molars based in the relation between LHMS/CIL. Normal molars vary in 0.18 to 0.23, is

found in *Neusticomys* and *Rheomys*. Reduced molars vary in 0.13 to 0.17, is shown in *Chibchanomys*, *A. leander* and *Ichthyomys*.

31. Upper third molars M3 — 3 states: 0 = Tricuspidated; 1 = Bicuspidated; 2 = Reduced (peg-like)

The third molar in ichthyomine species is reduced, but the number of cuspids is variable. Tricuspidate condition is found in *A. leander* and *R. underwoodi*. Bicuspidate molars are shown in *Ichthyomys*, *R. mexicanus*, *R. raptor*, *R. thomasi*, *C. trichotis* and Peruvian forms of *C. orcesi*. Reduced third molar peg-like is found in Ecuadorian forms of *C. orcesi*, and *Neusticomys*.

32. Complexity in molars m3 — 2 states: 0 = two distinct enameled lobes; 1 = simple peglike tooth

We follow to Voss (1988). The m3 with two distinct enameled lobes, the entoconid-hypoconid cusp pair comprising a large posterior moiety of the tooth, present and small in *A. leander*, *C. trichotis*, *Incanomys*; present and developed in *Ichthyomys* and *Rheomys*. The m3 as a simple tooth, the entoconid-hypoconid cusp pair absent, reduced to a small conule, is found in *Neusticomys*.

33. Posterolophs — 3 states: 0 = In M1 and M2; 1 = In M1 only; 2 = Absent

We follow to Salazar-Bravo et al. (2023). This trait describes the presence or absence of posterolophs in the upper molars. Posterolophs present in both M1 and M2, condition found in *C. trichotis*, Peruvian forms of *N. orcesi* and *Rheomys*. Posterolophs present in M1 only, is found in *A. leander* and *Daptomys*. Posterolophs absent in *Ichthyomys*, *Neusticomys*.

34. Posterolophids — 2 states: 0 = In m1 and m2; 1 = Absent

We follow to Salazar-Bravo et al. (2023). This character distinguishes the presence or absence of posterolophids in m1 and m2. Posterolophids in m1 and m2 found in *A. leander*, *C. trichotis*, *Incanomys*, *N. monticolus* and *N. vossi*. Absent in *Ichthyomys*, *N. orcesi*, *Daptomys* and *Rheomys*.

35. Omohyoid muscle — 2 states: 0 = Present; 1 = Absent

We follow the character 15 of Voss (1988), also followed by Salazar-Bravo et al. (2023). The omohyoid muscle is absent in *A. leander* and *R. mexicanus*.

36. Gastric glandular epithelium — 2 states: 0 = Hemiglandular; 1 = Reduced glandular zone

We follow to Carleton (1973) and Salazar-Bravo et al. (2023). This trait describes the anatomical characters of glandular gastric epithelium in ichthyomyines. Unilocular stomach with glandular epithelium present between the great curvature to antrum in position opposite incisura angularis, feature present in *Neusticomys* and *Chibchanomys*; unilocular stomach with the reduced glandular zone in the great curvature, condition present in *Ichthyomys*, *Rheomys* and *Anotomys*.

37. Gall bladder — 2 states: 0 = Present; 1 = Absent

We follow to Voss (1988) and Salazar-Bravo et al. (2023). The gall bladder absents in *Ichthyomys*.

38. Bacular cartilage — 3 states: 0 = Tridigitate and small; 1 = Unidigitate; 2 = Tridigitate with calcified core

In this character distinguishes the variation in development of bacular cartilage in gland penis (Hooper and Musser 1964, Voss and Linzey 1981, Voss 1988). Bacular cartilage is tridigitate when the medial digit larger than the lateral two but not grossly swollen nor containing a calcified center in *Ichthyomys*, *Neusticomys* and *Chibchanomys*. Bacular cartilage is unidigitate when it presents as a single digit, this state is found in *Anotomys*. Bacular cartilage is tridigitate with the medial digit grossly swollen with a well-defined calcified core, this state is restricted to *Rheomys*.

Table S3. List of GenBank accession numbers for molecular data used in this study.

Species	Cyt <i>b</i>	IRBP	RAG1
<i>Anotomys leander</i>	MW148637	MW148683	MW148706
<i>Chibchanomys trichotis</i>	MW148642	MW148687	
<i>Daptomys peruviansis</i>	MW148664	MW148697	MW148717
<i>Daptomys mussoi</i>	MW148657		
<i>Daptomys ferreirai</i>	MW148654	MW148692	MW148710
<i>Daptomys oyapocki</i>	MW148661	MW148695	MW148714
<i>Ichthyomys hydrobates</i>	MW148645	MW148688	
<i>Ichthyomys orientalis</i>	MW148646		
<i>Ichthyomys stolzmanni</i>	MW148648	MW148689	MW148708
<i>Ichthyomys tweedii</i>	MW148651	MW148690	
<i>Incanomys mayopuma</i> 1	MW148639	MW148685	MW148707
<i>Incanomys mayopuma</i> 2	MW148640	MW148686	
<i>Incanomys undescribed</i>	PP094261		
<i>Neusticomys vossi</i>	KF359513	KR105608	MW148719
<i>Neusticomys orcesi</i>	MW148641		
<i>Neusticomys monticolus</i>	KF359515	KR105606	MW148712
<i>Rheomys mexicanus</i>	MW148678	MW148702	MW148719
<i>Rheomys raptor</i>	KF359512	AY163635	MF097935
<i>Rheomys thomasi</i>	MW148682	MW148705	MW148721

Table S4. Genetic distances were estimated with the Kimura two parameters evolution model (Kimura 1980) of Ichthyomyini species, based on cytochrome *b*, genetic distance (below the diagonal) and standard error (upper diagonal).

B	Species: CYT b \ IRBP	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1	<i>A. leander</i> CTUA 2556		0.028	0.026	0.026	0.023	—	0.023	0.025	0.033	0.024	0.029	0.027	0.026	0.023	0.021	0.023	0.021	—
2	sp.nova MUSA13864	0.255		0.002	0.014	0.021	—	0.022	0.030	0.022	0.017	0.014	0.015	0.017	0.021	0.020	0.021	0.015	—
3	sp.nova MUSA18964	0.255	0.000		0.012	0.020	—	0.020	0.029	0.020	0.015	0.012	0.013	0.017	0.019	0.019	0.020	0.013	—
4	<i>N. orcesi</i> MEPN 12230	0.256	0.163	0.163		0.016	—	0.017	0.022	0.011	0.014	0.007	0.001	0.015	0.018	0.016	0.018	0.012	—
5	<i>I. hydrobates</i> MHN-UCa 1439	0.259	0.188	0.188	0.182		—	0.004	0.005	0.024	0.016	0.019	0.018	0.018	0.008	0.007	0.010	0.012	—
6	<i>I. orientalis</i> MECN 4914	0.263	0.180	0.180	0.190	0.042		—	—	—	—	—	—	—	—	—	—	—	—
7	<i>I. stolzmanni</i> MUSA18919	0.250	0.203	0.203	0.201	0.066	0.078		0.007	0.025	0.016	0.017	0.018	0.018	0.008	0.007	0.008	0.013	—
8	<i>I. tweedii</i> TK182286	0.278	0.182	0.182	0.198	0.135	0.128	0.125		0.023	0.021	0.023	0.023	0.024	0.011	0.011	0.013	0.015	—
9	<i>D. ferreirai</i> M150	0.284	0.153	0.153	0.157	0.204	0.196	0.212	0.202		0.024	0.012	0.012	0.026	0.024	0.021	0.024	0.018	—
10	<i>N. monticolus</i> QCAZ 6446	0.231	0.155	0.155	0.040	0.175	0.187	0.189	0.194	0.151		0.015	0.014	0.007	0.012	0.015	0.015	0.007	—
11	<i>D. oyapocki</i> T5760	0.262	0.163	0.163	0.147	0.200	0.191	0.197	0.221	0.049	0.153		0.008	0.017	0.020	0.017	0.018	0.012	—
12	<i>D. peruviansis</i> MUSA 12657	0.275	0.155	0.155	0.148	0.194	0.186	0.201	0.194	0.030	0.146	0.045		0.016	0.019	0.016	0.019	0.013	—
13	<i>N. vossi</i> QCAZ 7830	0.277	0.148	0.148	0.085	0.198	0.198	0.216	0.193	0.168	0.087	0.162	0.148		0.014	0.016	0.017	0.011	—
14	<i>R. mexicanus</i> MZFC13624	0.312	0.209	0.209	0.202	0.176	0.179	0.174	0.164	0.192	0.199	0.220	0.180	0.203		0.008	0.009	0.014	—
15	<i>R. raptor</i> KU159017	0.261	0.186	0.186	0.194	0.132	0.144	0.132	0.156	0.192	0.196	0.187	0.199	0.201	0.144		0.005	0.011	—
16	<i>R. thomasi</i> MUHNES 502168	0.287	0.188	0.188	0.211	0.162	0.170	0.164	0.164	0.218	0.201	0.234	0.232	0.218	0.186	0.091		0.014	—
17	<i>C. trichotis</i> JEC213	0.248	0.177	0.177	0.142	0.186	0.179	0.178	0.184	0.139	0.144	0.146	0.150	0.170	0.215	0.187	0.200		—
18	<i>D. mussoi</i> Colombia	0.273	0.165	0.165	0.154	0.199	0.200	0.207	0.195	0.036	0.151	0.048	0.012	0.157	0.191	0.194	0.241	0.154	
19	sp. nova SEP 40	0.270	0.121	0.121	0.200	0.204	0.209	0.218	0.201	0.190	0.189	0.195	0.178	0.181	0.207	0.198	0.216	0.193	0.183