Holcus pintodasilvae (Poaceae, Pooideae, Poeae), a New Species from the Island of Madeira (Portugal), and Notes on Macaronesian *Holcus*

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Abstract. A new species of *Holcus* L. (Poaceae), endemic to the island of Madeira (Portugal), is here described as *H. pintodasilvae* M. Seq. & Castrov. This new species is morphologically closely related to the Macaronesian endemics *H. rigidus* Hochst. ex Hochst. (Azores Archipelago) and *H. mollis* L. subsp. *hierrensis* Stierst. (El Hierro Island, in the Canary Islands) but differs by having the culm nodes light brown, the leaves with a glabrous sheath and subspreading lamina attenuated to an acuminate apex, the glumes blunt, shiny, glabrous (except in the minutely scabrous veins), the upper glume with proximal lateral veins, and the lemma of the upper floret with an awn ca. 4 mm, twice as long as the lemma. Chromosome counts in *H. pintodasilvae* are tetraploid ($2n = 28$). Morphology, ecology, biogeography, and conservation issues are discussed and related to other Macaronesian *Holcus* taxa. *Holcus pintodasilvae* was found as only three populations (totaling less than 20 individuals), and its IUCN conservation status is Critically Endangered (CR). A new specific status is proposed for *H. mollis* subsp. *hierrensis*, as *H. hierrensis* (Stierst.) Stierst. & M. Seq., based on its chromosome number and morphological identity.

Resumo. Descreve-se uma nova espécie, *Holcus pintodasilvae* M. Seq. & Castrov., pertencente ao género *Holcus* L. (Poaceae), endémica da Ilha da Madeira (Portugal). Morfologicamente próxima dos endemismos macaronésicos *H. rigidus* Hochst. ex Hochst. (Arquipélago dos Açores) e *H. mollis* L. subsp. *hierrensis* Stierst. (Ilha de Hierro, Arquipélago das Canárias) diferencia-se por possuir: nós de coloração castanha-clara; folhas com bainhas glabras e lâminas subpatentes atenuadas num ápice acuminado; glumas brilhantes, múticas e glabras (excepto nas nervuras que se apresentam escabriúsculas), gluma superior com as nervuras laterais proximais; lema da flor superior com arista ca. 4 mm (duas vezes o comprimento da lema). Incluem-se ainda resultados da contagem de cromossomas de *H. pintosilvae* ($2n = 28$). Discutem-se, e relacionam-se com outros taxa Macaronésicos do género *Holcus*, aspectos relativos à morfologia, ecologia, biogeografia e conservação. *Holcus pintodasilvae* foi encontrado em apenas três populações (num total de menos de 20 indivíduos), sendo o seu estado de conservação IUCN está criticamente ameaçado (CR). Propõe-se ainda a combinação *H. hierrensis* (Stierst.) Stierst. & M. Seq. baseada na sua identidade morfológica e número cromossomático.

Key words: Gramineae, *Holcus*, IUCN Red List, Macaronesia, Madeira, Poaceae, Portugal.

In a recent revision of the genus *Holcus* L. (Menezes de Sequeira, 2004), nine species, five subspecies, one \hybrid, and one variety were recognized. More recently, *H. azoricus* M. Seq. & Castrov. was described as an Azores endemic of possible hybrid origin (Menezes de Sequeira & Castroviejo, 2007). The genus *Holcus* is restricted to the Old World, but the human-mediated expansion of *H. lanatus* L. and *H. mollis* L. subsp. *mollis* give *Holcus* a worldwide distribution (Menezes de Sequeira, 2004; Menezes de Sequeira & Castroviejo, 2006). Within the genus, five taxa are Iberian Peninsula endemics (*H. grandiflorus* Boiss. & Reut., *H. gayanus* Boiss., *H. caespitosus* Boiss., *H. reuteri* Boiss., and *H. annuus* C. A. Mey. subsp. *duriensis* (P. Silva) Franco & Rocha Afonso), one taxon is a South African Cape Province endemic (*H. annuus* subsp. *setiger* (Nees) M. Seq. & Castrov.), and three are Macaronesian endemics (*H. rigidus* Hochst. ex Hochst., *H. mollis* subsp. *hierrensis* Stierst., and *H. azoricus*). Recent molecular data seem to group *Holcus* within the subtribe Holcinae of Poeae, possibly with the genus *Vahlodea* Fr. (Soreng et al., 2003; Quintanar et al., 2007), rather than sharing a common ancestor within Aveneae (e.g., *Deschampsia* P. Beauv. and *Hierochloe* R. Br.), as previously

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suggested by Clayton and Renvoize (1986) and Watson and Dallwitz (1994).

The Madeira Archipelago is composed of two main islands, Porto Santo (dating back to ca. 20 Ma) and Madeira (less than 5.6 Ma; Ribeiro et al., 2005). The island of Madeira is situated between 32°38′ and 32°52′N and 16°39′ and 17°16′W, approximately 600 km northwest of the western African coast, with a land area of 737 km². The vascular flora of Madeira includes 1137 taxa (species and subspecies), of which 96 taxa are exclusive endemics, with the Poaceae being the most diverse plant family (133 taxa, with six endemics, including *Agrostis obtusissima* Hack., *Anthoxanthum maderense* Teppner, *Deschampsia argentea* (Lowe) Lowe, *D. maderensis* (Hack. & Bornm.) Buschm., *Festuca donax* Lowe, and *Koeleria loweana* Quintanar, Catalán & Castrov.; see Jardim & Menezes de Sequeira, 2008).

Hansen and Sunding (1979, 1993) and Cope (1994) listed *Holcus lanatus* for the islands of Madeira, Porto Santo, and the Selvagens and *H. mollis* for Madeira. Other authors, such as Menezes (1906, 1914), mention only *H. lanatus*. In fact, *H. lanatus* collections date back to the 19th century, and the species' expansion is clearly human-mediated through habitat change, but its arrival in the flora could have been prior to human settlement (Menezes de Sequeira, 2004; Jardim & Menezes de Sequeira, 2008), at least on marine cliffs and in humid habitats. Although Cope (1994) regarded *H. mollis* as native, and Robert Brown (1773–1858) listed the plant in his account of Madeiran species (Britten, 1904) (possibly in error for *H. lanatus*, not included on the list), and recently Vieira (2002), based on Cope (1994), excluded this species in his account of the exotic plants of Madeira, references to *H. mollis* are very recent. The first known specimen of *H. mollis* was collected by Costa with the notes "Santa, no Pôrto do Moniz, vii–1934, Costa, MADM," possibly corresponding to a human-mediated introduction, established during the massive afforestation that took place in the first half of the 20th century (Menezes de Sequeira et al., 2007; Jardim & Menezes de Sequeira, 2008).

The Macaronesian endemics *Holcus rigidus* and *H. mollis* subsp. *hierrensis* (Stierstorfer, 2001; Menezes de Sequeira & Castroviejo, 2007) are small chamaephytes with aboveground branching, whereas *H. azoricus* is a rhizomatous hemicryptophyte of putative hybrid origin (*H. rigidus* × *H. lanatus;* Menezes de Sequeira & Castroviejo, 2007). The morphological segregation of Macaronesian endemics belonging to the genus *Holcus*, namely the chamaephyte habit, clearly suggests that a specific rank should be applied to these endemics, and accordingly, a new status is proposed for *H. mollis* subsp. *hierrensis*.

1. **Holcus hierrensis** (Stierst.) Stierst. & M. Seq., comb. et stat. nov. Basionym: *Holcus mollis* L. subsp. *hierrensis* Stierst., Feddes Repert. 112: 49. 2001. TYPE: Spain. Canary Islands: El Hierro, SE of Eremita de la Caridad, 1270 m.s.m., UTM 28RBR065742, N-facing slope, Vitrands, blocks of lava, 10 July 1999, *Ch. Stierstorfer s.n.* (holotype, B-100006407; isotypes, M not seen, TFC not seen).

Apart from the new status proposed above as *Holcus hierrensis* for the El Hierro endemic, recent fieldwork demonstrated the presence of a new species restricted to Madeira. Plants of this new species found in the higher mountains of Madeira show a typical Macaronesian chamaephyte habit, but they also show very distinct characters, and accordingly a new species is described herein.

2. **Holcus pintodasilvae** M. Seq. & Castrov., sp. nov. TYPE: Portugal. Madeira: Pico do Arieiro, ca. Pico do Gato, 1670 m.s.m., exp. N, 32°44′30.6″N, 16°56′17.4″, 16 July 2003, *M. Menezes de Sequeira 4368* (holotype, MA-807339). Figures 1–4.

Haec species inter congeneros chamaephyta rhizomatosa *Holco rigido* Hochst. ex Seub. et *H. hierrensi* (Stierst.) Stierst. & M. Seq. similis, sed ab eis nodis brevissimis atque dilute castaneis, foliorum vaginis laminisque glabrescentibus laminis subpatentibus atque attenuato-acuminatis, glumis nitentibus fere glabris (praeter nervos carinamque minute scabros), gluma superiore nervis lateralibus propius ad costam quam marginem et flosculi superioris lemmate in aristam ca. 4 mm longam (lemmate duplo longiorem) desinente differt.

Rhizomatous chamaephyte (Figs. 1A, 3B), with a slender ramified rhizome; culms 55–79 cm tall, branched above ground; nodes light brown, 0.5–1 mm, glabrous (Figs. 1B, 3C). Leaves rigid, subpungent, subspreading; sheath glabrous (Fig. 1B), median leaves with sheath 3.8–6.6 cm, upper leaves with sheath 7–11 cm; lamina minutely scabrous (Fig. 2A, B), linear-lanceolate (Fig. 3A), flat to convolute, persistent, attenuate to an acuminate apex, median leaves 57.7–104.2 × 2.6–5.1 mm, upper leaves 11.8–35.7 × 8.4–15.3 mm; ligule 2–2.8 mm (Figs. 1B, 3D), asymmetric with long asymmetrical teeth, membranous, glabrous except for the short ciliate margins (hairs 14–26 μm), shortly toothed. Panicle (Figs. 3A, 4) 4.7–7.7 cm, whitish and shiny (Fig. 1C), few-flowered (ca. 100), somewhat compact (spike-like), regular in outline; branches scabrous with

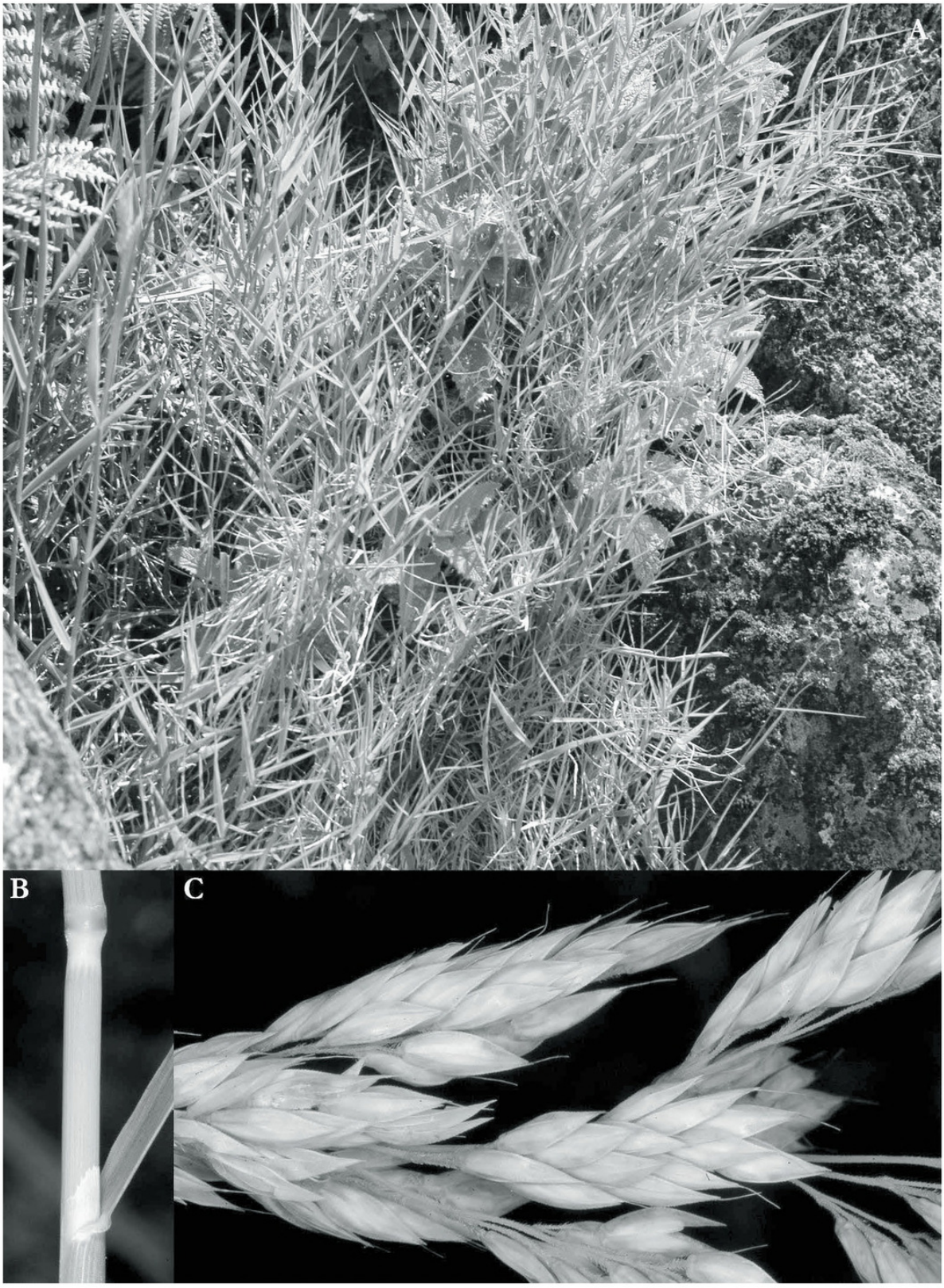

Figure 1. *Holcus pintosasilvae* M. Seq. & Castrov. —A. Habit. —B. Detail of the node, stem, and ligule. —C. Detail of the inflorescence. Photographs taken in Madeira in Bica da Cana by M. Sequeira on 26 June 2007.

acicular hairs. Spikelets (Figs. 1C, 3E, 3F) 4.5–6.4 mm, falling entire, with a well-marked abscission zone at the base of the glumes, with 2 florets; glumes thinly papery, shiny, slightly unequal, enclosing the flowers, apex acuminate, blunt, keeled, both keel and nerves minutely scabrous; lower glume 4.5–5.8 × 1.7–2.1 mm, 1-nerved (Fig. 3G), lamina glabrous, shiny, keel scaberulous, prickle hairs 19–109 µm,

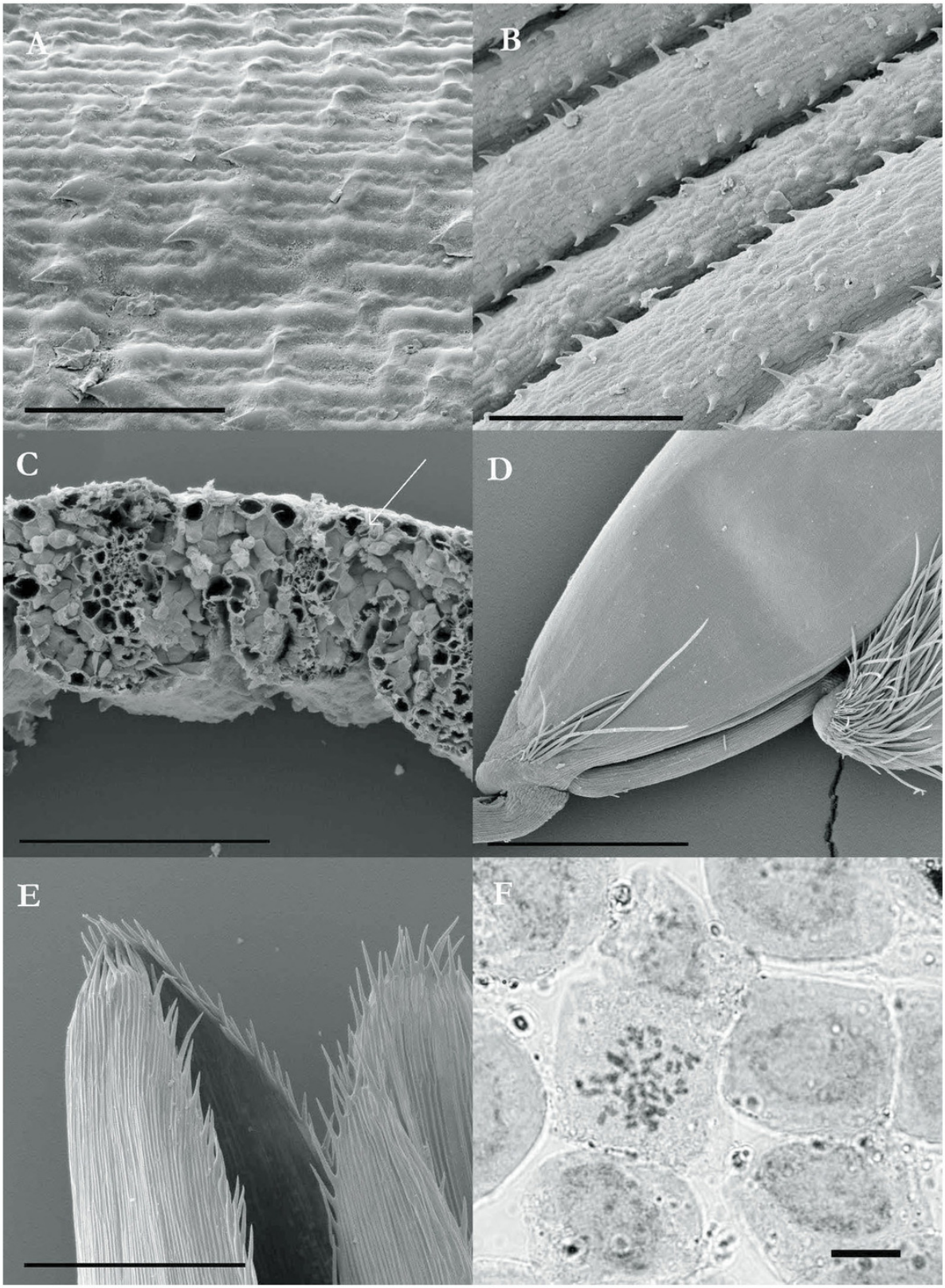

Figure 2. Scanning electron micrographs (taken on a JEOL-TSM T330A) of *Holcus pintodasilvae* M. Seq. & Castrov. (*M. Menezes de Sequeira 4368*, holotype, MA 807339). —A, B. Leaf adaxial surfaces. —C. Leaf section showing sclerenchyma cells (arrow). —D. Median rachis and florets, with callus showing indumentum. —E. Lower floret, showing lemma and palea apex (taken from the paratype *M. Sequeira 4630B*, MADM). —F. Cells in somatic metaphase, $2n = 28$, prepared from rhizomes cultivated at MADM. Scale bars: A = 100 μm; B, E = 300 μm; C = 200 μm; D = 500 μm; F = 10 μm.

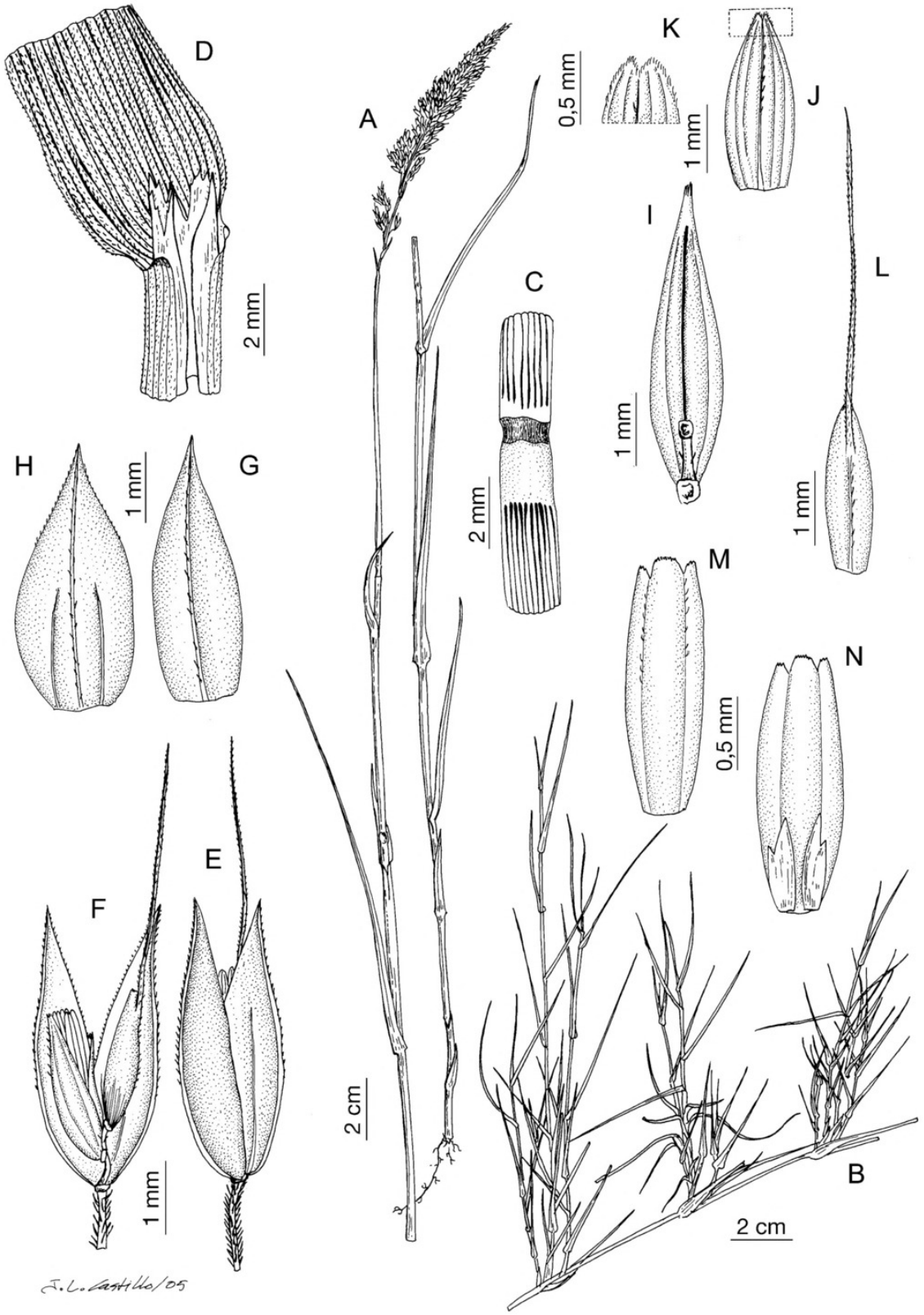

Figure 3. *Holcus pintodasilvae* M. Seq. & Castrov. —A. Culm and panicle. —B. Branching habit. —C. Culm node. —D. Detail of ligule at sheath and blade junction. —E. Spikelet. —F. Longitudinal section of the spikelet. —G. Lower glume. —H. Upper glume. —I. Lower floret. —J. Lemma of the lower floret. —K. Apex of the lower lemma. —L. Upper lemma. —M. Palea of lower floret (dorsal view). —N. Palea of lower floret (ventral view) and lodicules. Drawn from the holotype *M. Menezes de Sequeira 4368* (MA-807339).

Figure 4. *Holcus pintodasilvae* M. Seq. & Castrov. Photograph of the holotype *M. Menezes de Sequeira 4368* (MA-807339).

apex acuminate, blunt; upper glume 4.4–6.1 × 2.3–3 mm, 3-nerved (Fig. 3H), lateral veins proximal, keel scaberulous, prickle hairs 23–82 µm, apex acuminate blunt; rachilla disarticulating, extending beyond the distal floret, divided in 3 parts: proximal segment short, 0.33–0.53 mm (between the glumes and the first floret), curved, glabrous; the median segment (between the florets) longer, 0.6–0.75 mm, straight, glabrous to puberulous (Figs. 2D, 3F); distal segment (above the upper floret), rudimentary, ca. 0.1 mm,

villous and setose. Lower floret hermaphroditic; callus very short, glabrous; lemma cartilaginous (Fig. 3J), 2.2–2.6 mm, shortly puberulous on the keel, deeply emarginate, short ciliate (Figs. 2E, 3K); palea 2.1–2.5 mm, membranous, trilobed with the lateral lobes as long as or slightly shorter than the median (entire) (Figs. 2E, 3M, 3N), keels (2) pubescent, included within the lemma, adherent to the caryopsis, lodicules ca. 0.7 mm (Fig. 3N), with a turgescent circular-elliptic base, glabrous, acuminate, symmetric, with a short lateral tooth, ca. 35 μm, inserted in the distal 2/3; stamens 3 (falling before those of the upper floret), anthers ca. 1.2–1.3 mm long, blunt. Upper floret staminate; callus 0.1–0.2 mm long, pear-shaped to rounded, pubescent with hairs 36–915 μm long; lemma 2.2–2.6 mm (Fig. 3L), puberulous, emarginate, with a dorsal awn 3.3–4.1 mm, geniculate, inserted in the distal 2/3 to 3/4 of the lemma; palea 2–2.4 mm, 2-nerved, shortly trilobed, pubescent; anthers ca. 1.3 mm long, blunt.

Leaf anatomy. Although Watson and Dallwitz (1994) indicated that all sclerenchyma cells in *Holcus* are associated with vascular bundles, the presence of sclerenchyma cells close to the abaxial blade surface not associated with the vascular bundles was indicated by Menezes de Sequeira (2004) exclusively for *H. rigidus*. However, *H. pintodasilvae* also presents sclerenchyma cells beneath the abaxial surface that are not associated with the vascular bundles (Fig. 2C). Leaf rigidity results from the presence of these sclerenchyma cells not associated with the vascular bundles and also from the presence of sclerenchyma in all the vascular bundles.

Chromosome counts. Due to the scarcity of individuals found in nature, chromosome counts, in accordance with Menezes de Sequeira and Castroviejo (2007), were performed in only three rhizome portions collected (non-destructive sampling) from three different plants that were then grown at the University of Madeira gardens. Chromosome counts on *Holcus pintodasilvae* correspond to a tetraploid genome, with $2n = 28$ (Fig. 2F). Due to chromosome pairing, an autopolyploid origin is hypothesized, possibly close to *H. mollis*.

Polyploidy is common among endemic Macaronesian grasses, for example, the tetraploid Madeiran *Dactylis smithii* Link subsp. *hylodes* P. F. Parker (Parker, 1972) and the high 25-ploid Madeiran *Koeleria loweana* (Quintanar et al., 2006). However, Menezes de Sequeira et al. (2009) have shown that this is not the case for native *Festuca* L., where all five Macaronesian species were diploid. *Holcus pintosasilvae* is a tetraploid taxon, as are *H. rigidus* (Menezes de Sequeira, 2004; Menezes de Sequeira & Castroviejo, 2007) and *H. hierrensis* (Strierstorfer, 2001). These Macaronesian endemic and tetraploid *Holcus* species correspond to apoendemics, following the classification proposed by Favarger and Contandriopoulos (1961) and Contandriopoulos (1988). They are presumed to have evolved from one or several colonization events from *H. mollis* diploid paleoendemic-patroendemic (see Favarger & Contandriopoulos, 1961; Contandriopoulos, 1988) Iberian populations, as suggested by Menezes de Sequeira (2004) and Menezes de Sequeira and Castroviejo (2007). This was in agreement with Boissier and Reuter (1852), who considered Hispania (the Iberian Peninsula) and part of Mauritania (northwestern Africa) to be the dispersal center for the genus *Holcus*. References to diploid *H. mollis* plants, in fact, are restricted to Portugal (Fernandes & Queirós, 1969) and Bulgaria (Walter, 1980), possibly corresponding to a formerly continuous Ibero–Turanian distribution affected by Pleistocene glaciations (Menezes de Sequeira, 2004).

Distribution and habitat. *Holcus pintodasilvae* is a very rare endemic found so far in only three populations on Madeira. As has been stated by Menezes de Sequeira et al. (2008) for the recently described *Teucrium francoi* M. Seq., Capelo, J. C. Costa & R. Jardim, there was a positive impact on population numbers and size caused by the removal of goats from the higher mountains of Madeira (Sousa, 2003), and this was possibly related to the discovery of *H. pintodasilvae*. However, the new taxon could have been overlooked by previous collectors, who could have confused it with *Anthoxanthum maderense* or *Agrostis obtusissima*, which have a similar growth form. The chamaephytic habit is strongly correlated with susceptibility to damage from grazing, and the rarity of *H. pintodasilvae* could therefore be related to the long-term effects of grazing by goats introduced immediately after human colonization in the 15th century, which caused massive landscape changes (Menezes de Sequeira et al., 2007). Although Silva et al. (unpublished) noted a quick recovery of the formerly rare plant endemics after goats were removed, they also observed that this recovery was followed by a strong resurgence of invasive plants such as *Cytisus scoparius* (L.) Link subsp. *scoparius*, with negative impacts on chamaephytic endemics, such as *H. pintodasilvae*, by habitat reduction. *Holcus pintodasilvae* occurs in the *Armerio maderensis–Parafestucetum albidae* and *Teucrio francoi–Origanetum virentis* plant communities (Capelo et al., 1999; Costa et al., 2004).

Table 1. Diagnostic characters of *Holcus pintodasilvae* M. Seq. & Castrov. and related taxa.

Characters	*H. azoricus*	*H. rigidus*	*H. mollis*	*H. hierrensis*	*H. reuteri*	*H. pintodasilvae*
Life form	rhizomatous hemicryptophyte, with a short slender rhizome	rhizomatous chamaephyte, with a long slender rhizome	rhizomatous hemicryptophyte, with a long slender rhizome	rhizomatous chamaephyte, with a long slender rhizome	rhizomatous hemicryptophyte, with enlarged rhizome	rhizomatous chamaephyte, with a long slender rhizome
Nodes	dark, pubescent	dark, glabrous	sometimes dark, bearded	sometimes dark, bearded	sometimes dark, bearded	light brown, glabrous
Leaves	not spreading, subpungent; sheath pubescent to villose	spreading, pungent; sheath glabrous	not spreading or pungent; sheath pubescent to villose	not spreading or pungent; pubescent toward the base	not spreading or pungent; sheath glabrous, rarely pubescent toward the base	subspreading, subpungent; sheath glabrous
Ligules	symmetric, surface puberulous to pubescent	asymmetric, surface glabrous to puberulous	symmetric, surface glabrous to puberulous	symmetric, surface puberulous	symmetric, surface pubescent (rarely glabrous)	asymmetric, surface glabrous
Leaf blades	scaberulous to pubescent, linear-lanceolate, attenuate in subacuminate apex	scaberulous, linear-lanceolate, attenuate in acuminate and rigid apex	pubescent, linear to oblong-lanceolate, attenuate in acute apex	pubescent, linear to oblong-lanceolate, attenuate in acute apex	pubescent, linear to oblong-lanceolate, attenuate in acute apex	scaberulous, linear-lanceolate, attenuate to acuminate apex
Glumes	equivalent, usually mucronate	slightly different, seldom mucronate	slightly different to unequal, blunt	slightly different, blunt	slightly different to unequal, blunt	slightly different, blunt
Upper glume of florets	lateral veins usually proximal	lateral veins usually marginal	lateral veins proximal	lateral veins marginal	lateral veins proximal	lateral veins proximal
Glume indumentum	keel, veins, and lamina minutely scabrous to pubescent	keel, veins, and lamina minutely scabrous	keel and veins with long hairs; lamina minutely scabrous to pubescent	keel and veins with long hairs; lamina minutely scabrous to pubescent	keel and veins with long hairs; lamina minutely scabrous to pubescent	keel and veins minutely scabrous; lamina glabrous, shiny
Inferior palea of florets	1.7–2.5 mm, trilobed, lateral lobes shorter than the median	2.1–2.4 mm, trilobed, lateral lobes much shorter than the median (emarginate)	1.7–2.6 mm, trilobed, lateral lobes much shorter than the median	1.9–2.1 mm, trilobed, lateral lobes slightly shorter than the median	2–2.4 mm, trilobed, lateral lobes shorter than the median	2.1–2.5 mm, trilobed, lateral lobes as long as or slightly shorter than the median (entire)
Median segment of rachilla	glabrous to pubescent	puberulous to pubescent	puberulous to villose and setose	villose and setose	puberulous to pubescent	glabrous to puberulous
Lema of upper distal staminate floret	1.9–2.6 mm	2.4–2.9 mm	1.9–3 mm	1.9–2.5 mm	2.3–2.7 mm	2.2–2.6 mm
Awn of the upper staminate floret	1.9–3.9 mm, hooked or geniculate	2.2–3.9, geniculate	0.2–5.5 mm, geniculate	3.1–3.7 mm, geniculate	4.3–5.2 mm, geniculate	3.3–4.1 mm, geniculate

IUCN Red List category. *Holcus pintodasilvae* should be considered as Critically Endangered (CR, C2a(i,ii)D; IUCN, 2001) since only three populations are known (less than 20 individuals). These populations occupy an area of less than 200 m^2 and appear to have been reduced in size by past grazing (the plant habit and the fact that they were discovered after grazers were removed seem to support this hypothesis).

Phenology. Flowering in *Holcus pintodasilvae* occurs from May to July.

Etymology. The new species epithet is dedicated to António Rodrigo Pinto da Silva (1912–1992), a respected Portuguese botanist who also studied the flora of Madeira and contributed to the foundation of the Madeira Botanical Garden.

Discussion. Several examples of perennial chamaephyte grasses occur on Madeira. The most spectacular is *Koeleria loweana* [≡ *Parafestuca albida* (Lowe) E. B. Alexeev]. *Dactylis smithii* and *Anthoxanthum maderense* also display the perennial chamaephyte habit that characterizes *Holcus pintodasilvae*. *Holcus pintodasilvae* shares a similar morphology with the *H. mollis* species complex (*H. mollis*, *H. reuteri*, *H. hierrensis*, *H. rigidus*, and also partially with *H. azoricus*). These characteristics include the rhizomatous, perennial habit (Figs. 1A, 3B, 4), deciduous spikelets, blunt glumes, two florets, with the lower floret hermaphroditic and awnless but the upper floret staminate with a long geniculate awn. Study of fertile specimens of the new species indicates an overall resemblance with both *H. rigidus* and *H. hierrensis*. However, other characteristics clearly separate *H. pintodasilvae* from morphologically closely related species (cf. Table 1). *Holcus pintodasilvae* and *H. rigidus* have rigid stems and leaves, but *H. pintodasilvae* differs from other taxa by its short and congested branching of the culm, the glabrous, light brown, and very short nodes (Fig. 1B), the less-pointed leaves (not so clearly spreading), the glabrescent sheaths and lamina (only extremely short, scabrous prickle hairs are present), and the strongly asymmetric ligules. Unlike *H. rigidus* and *H. hierrensis*, *H. pintodasilvae* presents proximal nerves in the upper glume, and it differs from all other species in the *H. mollis* complex by presenting shiny glumes.

Paratypes. PORTUGAL. **Madeira:** Bica da Cana, 1500 m.s.m., 90°E, 26 June 2005, *M. Menezes de Sequeira 4625B* (MADM); Circa Pico do Areeiro, Manga dos Vómitos, 1650 m.s.m., NNW, 26 June 2005, *M. Menezes de Sequeira 4630B* (MADM; MO); Bica da Cana, nas fissuras terrícolas do afloramento rochoso perto do posto florestal, 5 Aug. 2004, *M. Menezes de Sequeira & P. Catálan 4538* (MADM); Achada do Teixeira, subida para o Pico Ruivo, 1703 m.s.m., exp. N, 32°45.8′N, 16°55.9′W, 18 July 2010, *M. Menezes de Sequeira & R. Piñeiro 6687* (MADM).

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