UNIVERSIDAD AUTÓNOMA DE MADRID
FACULTAD DE CIENCIAS
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Estudios de taxonomía integrativa en el género *Orthotrichum* Hedw., subgénero *Pulchella* (Schimp.) Vitt (Bryophyta)

Rafael Medina Bujalance
Memoria de Tesis Doctoral

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Memoria para optar al grado de Doctor en Biología que presenta
Rafael Medina Bujalance

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Francisco Lara García y Ricardo Garilleti Álvarez.

Dr. Vicente Mazimpaka Nibarere   Dr. Francisco Lara García   Dr. Ricardo Garilleti Álvarez

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A mis padres

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Índice

I.

Introducción

“Aquí hay varias cosas”

Proverbio taxonómico

Relevancia de la taxonomía integrativa

La delimitación y descripción de las especies de organismos, o taxonomía alfa (Davis & Heywood, 1963), constituye una de las tareas más ingentes de la biología. Si bien el número de especies conocidas en la actualidad puede situarse alrededor de los 1.35 millones (Bisby et al., 2011), no existe un consenso acerca del total. Pese a todo, en líneas generales, las estimaciones coinciden en postular que la mayor parte de la diversidad específica de la biosfera está pendiente de descripción (Mora et al., 2011). La taxonomía alfa, al delimitar las "unidades de medida" de otras disciplinas, cumple un papel clave y fundamental para el desarrollo de las ciencias biológicas (Wilson, 2004). Su relevancia afecta de forma especialmente directa a campos tan significativos como la ecología (Wanntorp et al., 1990), la biogeografía (Santos & Amorim, 2007) o la biología de la conservación (Vane-Wright, 1996; Williams et al., 1991) y una mala praxis taxonómica produce errores en cadena en los campos que en ella se fundamentan (Bortolus, 2008).

Durante la pasada década, la taxonomía alfa se ha visto inmersa en una crisis (Wilson, 2004; Wheeler, 2004) relacionada con varios factores. Entre ellos está la crítica recurrente a la subjetividad de la delimitación específica, que en la práctica se fundamenta con frecuencia en un concepto de especie "puramente morfológico" y por lo tanto "arbitrario" y dependiente del criterio experto del especialista (McDade, 1995). En estrecha relación con ello se encuentra la posibilidad alternativa de emplear la gran cantidad de información filogenética contenida en las secuencias de macromoléculas, un recurso que, si bien es conocido desde hace tiempo (Zuckerkandl & Pauling, 1965), se ha generalizado con gran éxito a raíz del abaratamiento de los costes de amplificación y secuenciación de ADN, al hacerlo accesible a un mayor número de laboratorios de investigación. Ello ha llevado incluso a pensar en el desarrollo de un sistema automatizado de identificación de especies a partir de secuencias concretas del genoma (Hebert & Gregory, 2005; Savolainen et al., 2005), conocido como *barcoding*, que supuestamente permite también reconocer rápidamente taxones nuevos para la ciencia. Además, las técnicas de secuenciación de última generación (Ansorge, 2009; Schuster, 2008) han conseguido que el estudio cotidiano de genomas prácticamente completos sea una realidad.

Este conjunto de circunstancias ha llevado a pensar a una parte de la comunidad científica que la figura del taxónomo especializado ya no es necesaria, pues su labor de proveer "opiniones" más o menos formadas sobre la delimitación de especies puede ser sustituida por los usuarios finales de las mismas si cuentan con los medios adecuados (Godfray, 2007).

Las críticas arriba mencionadas también han recibido las réplicas correspondientes. Son muchas las voces que se han alzado en defensa del prestigio y la necesidad de la taxonomía en un momento tan crítico para la conservación de la biodiversidad como lo es el actual (Agnarsson & Kuntner, 2007; Carvalho et al., 2008; Wilson, 2004) y que han denunciando el agravio comparativo que sufre el trabajo descriptivo en la valoración de las publicaciones mediante índices de impacto (Werner, 2006).

El hecho de que la búsqueda de una definición teórica rigurosa del concepto de especie sea un debate secular (Wilkins, 2009b) aparentemente estéril para muchos, ha contribuido a reforzar una impresión de arbitrariedad y desconcierto ilustrada por la coexistencia de hasta 26 definiciones modernas (Wilkins, 2009a). Sin embargo, aunque el debate no está exento de interés epistemológico, no hay motivos por los que deba afectar al desarrollo empírico de la taxonomía (Pigliucci, 2003), especialmente si se distingue correctamente entre la conceptualización y la delimitación (Queiroz, 2007). Pese a todo, se mantiene la cuestión de la subjetividad del criterio experto del taxónomo al escoger los caracteres, con lo que se hace deseable una fuente de información independiente para contrastar la hipótesis morfológica.

Resulta relevante destacar que, al contrario de lo que podría pensarse, en la praxis de la filogenia molecular también son muchas las decisiones que se toman que dependen del criterio experto del investigador. Entre ellas figuran la propia selección de marcadores y de su número adecuado, la asunción de que las secuencias son ortólogas, el correcto alineamiento de las mismas, la elección del modelo de sustitución y del método estadístico empleado para la reconstrucción filogenética y, por supuesto, la hipótesis subyacente en la identificación de todos y cada uno de los especímenes de los que se extrae material genético. En todos estos pasos es posible que decisiones arbitrarias del investigador tengan consecuencias en los resultados obtenidos (Jenner, 2004; Kelchner, 2000; Vandamme, 2003; Werner et al., 2007), y aunque esta incertidumbre se asume como necesaria durante el proceso, con frecuencia se omiten valoraciones críticas. Por su parte, la aspiración de automatizar la identificación de todos los organismos mediante *barcoding* ha sido criticada por su reduccionismo en numerosas ocasiones (DeSalle, 2006; Ebach & Holdrege, 2005; Will et al., 2005). Por último, la delimitación de especies únicamente mediante estudios derivados de la filogenia molecular o la filogenómica no permite que este conocimiento sea accesible a una parte significativa de la sociedad en general y de la comunidad científica en particular (Mayo et al., 2008).

Culminar con éxito la labor de describir la diversidad específica aún desconocida dependerá en gran medida de cómo se solventen las imperfecciones de uno y otro tipo de aproximaciones. En el contexto que se ha mencionado, resulta evidente que la taxonomía basada en caracteres morfológicos y la reconstrucción filogenética basada en secuencias de ácidos nucleicos pueden beneficiarse de una iluminación recíproca (Steele & Pires, 2011) testando cada una la hipótesis aportada por la otra, y permitiendo valorarlas críticamente. La necesidad de integrar y sintetizar diversas fuentes de información útil para la delimitación y descripción de especies desemboca en la llamada taxonomía integrativa[1] (Dayrat, 2005), que se define como "la ciencia que pretende delimitar las unidades de la diversidad de la vida a partir de perspectivas múltiples y complementarias (filogeografía, morfología comparada, genética de poblaciones, ecología, biología del desarrollo, etología, etc)". El enfoque integrativo y la creciente accesibilidad a todo tipo de información taxonómica se postulan en estos momentos como la salida a la mencionada crisis, tanto por la posibilidad de ofrecer soluciones taxonómicas más sólidas como por hacerlas más inclusivas (Assis, 2009; Carvalho et al., 2008; Mayo et al., 2008).

Entre los organismos cuyo conocimiento podría verse especialmente beneficiado por este enfoque, encontramos aquellos cuyas particularidades taxonómicas o biológicas limitan las posibles fuentes de información o que no han recibido la misma atención que otros grupos estudiados de forma preferente, como los vertebrados o las angiospermas. Los briófitos constituyen un ejemplo de este tipo de organismos.

Particularidades de la delimitación de especies en briófitos

Los briófitos en sentido amplio (divisiones Marchantiophyta, Bryophyta y Anthocerotophyta) constituyen un grupo de plantas terrestres muy diversificado y representado en todo tipo de ecosistemas. Sus más de 17.000 especies descritas (Frey et al., 2009) convierten a los briófitos en las plantas con mayor diversidad específica después de las angiospermas. En su inmensa mayoría, las especies de briófitos se han delimitado y descrito siguiendo criterios morfológicos (Shaw, 2008). Sin embargo, su relativa sencillez estructural, a la vez que condiciona toda una serie de estrategias fisiológicas y ecológicas, convierte a estas plantas en linajes "austeros" desde un punto de vista morfológico (Bickford et al., 2007), en los que encontrar caracteres de utilidad taxonómica presenta dificultades añadidas. Lógicamente esta circunstancia afecta a la delimitación de especies.

Las posibles particularidades de los briófitos en este aspecto se han relacionado a menudo con la información biogeográfica de la que se disponía. Se considera como un hecho aceptado que las especies de briófitos presentan, generalmente, áreas de

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[1] La denominación empleada por Dayrat (*integrative taxonomy*) se encuentra ya bien establecida en la literatura biológica en inglés de la última década. Sin embargo, no parece haberse afianzado una traducción de este concepto en castellano. De entre otros posibles epítetos que serían válidos en este idioma (integradora, integrable,...), se ha preferido el uso del sufijo –iva por mantener el carácter activo y su significado "con la cualidad de" (cf. Fernández Ramírez, S. 1975. Derivados españoles en –ivo. *Archivum. Revista de la Facultad de Filología. Universidad de Oviedo* 25: 323–327).

distribución más extensas que las de las plantas vasculares, incluyendo frecuentes disyunciones transoceánicas (Medina et al., 2011; Vanderpoorten & Goffinet, 2009). Las hipótesis que se barajan para explicar este tipo de disyunciones en briófitos son la vicarianza provocada por la tectónica de placas (Schofield, 1988) o la dispersión a larga distancia (Frahm, 2009). En uno u otro caso se destaca, alternativamente, la capacidad de las especies de briófitos de mantenerse en una prolongada estasis evolutiva (Stenøien, 2008), o la alta conectividad genética entre distintas áreas, favorecida por sus propágulos microscópicos, que dificulta la especiación alopátrica (e.g. Shaw et al., 2003b).

No obstante, en numerosas ocasiones también se ha detectado el fenómeno contrario: lejos de ser uniformes, las grandes áreas disyuntas de algunos briófitos han manifestado una compleja estructura filogenética. Dado que se asumía la uniformidad morfológica de estas especies a lo largo de su área, gran parte de estos casos han pasado a considerarse complejos de especies crípticas (cf. Heinrichs et al., 2009 para una revisión detallada). En el caso de grandes áreas de distribución, esta circunstancia se ha podido explicar mediante procesos de especiación simpátrica, aunque también se han señalado supuestos casos de especiación críptica en simpatría en áreas relativamente restingidas (Fernandez et al., 2006).

La profusión de este tipo de investigaciones ha llevado a aceptar como algo evidente que, debido a la escasez de caracteres morfológicos que ofrecen al estudio, las morfoespecies de briófitos son especialmente vulnerables a la refutación mediante análisis filogenéticos (Vanderpoorten & Shaw, 2010). Es digno de mención que estas afirmaciones se hacen a pesar de que normalmente no se lleva a cabo una re-evaluación morfológica de la delimitación específica que se asume como premisa. Cuando ésta tiene lugar, aunque sea a posteriori, sí que pueden alcanzarse conclusiones sintéticas que lleven a la descripción formal de nuevas especies (e.g. Hedenäs et al., 2009; Szweykowski et al., 2005) o la recuperación de taxones previamente sinonimizados (e.g. Draper & Hedenäs, 2008; Renner et al., 2010). En este contexto es razonable sugerir que la aplicación de soluciones taxonómicas integrativas tiene el potencial de resultar especialmente beneficiosa en el caso de los briófitos, máxime en aquellos grupos o géneros en los que la delimitación específica presenta una especial complejidad.

Taxonomía y filogenia en *Orthotrichum*: algunos antecedentes

El género *Orthotrichum* Hedw. combina una elevada diversidad específica con una prolijidad taxonómica que se ha puesto de relieve en varias ocasiones. Es un género muy diverso, presente en todos los continentes y en un amplio espectro de ambientes, exceptuando algunos desiertos y las pluvisilvas tropicales (Lewinsky, 1993). Se trata además de un género con una prolongada historia botánica, que ya estaba incluido en *Species Muscorum* (Hedwig, 1801) y que, en el momento de la redacción de esta memoria (febrero de 2012), comprende 160 especies aceptadas (Goffinet et al., 2007; actualizado según Fedosov & Ignatova, 2010; Lara et al., 2007; 2009a; 2009b; Medina et al., 2008; 2011; Plášek et al., 2009), fundamentalmente epífitas y saxícolas.

Este elevado número de especies, unido a la relativa uniformidad del aspecto de estas plantas en su conjunto y la gran variabilidad intraespecífica de muchos de sus caracteres, son los responsables de que su delimitación específica haya atravesado oscilaciones muy llamativas. A lo largo del siglo XIX y hasta la mitad del siglo XX fue preponderante un criterio analítico que llevó a la descripción de muchas de las variaciones morfológicas como especies nuevas para la ciencia, incrementando el número de nombres publicados válidamente hasta alrededor de 600, de los cuales 232 correspondían a binomios, en principio, aceptados (Wijk et al., 1964). Para entonces, la identificación de especies de *Orthotrichum* ya era reconocida como una tarea particularmente espinosa y sobre la que era difícil poner de acuerdo incluso a distintos especialistas en briófitos (Moxley, 1937).

Por suerte, la briología pudo contar con la extraordinaria labor científica de Jette Lewinsky[2], que contribuyó de forma determinante al conocimiento integrado y sintético que tan necesario se había hecho. De entre sus numerosas publicaciones destacan las minuciosas revisiones del género que llevó a cabo para el África subsahariana (Lewinsky, 1978), Australasia (Lewinsky, 1984b), Sudamérica (Lewinsky, 1984a; 1987) y el sudeste asiático (Lewinsky, 1992), que culminaron en una reconocida sinopsis mundial (Lewinsky, 1993). En dicha sinopsis, Lewinsky acota a 116 el número de especies aceptadas y evalúa sintéticamente la utilidad taxonómica de los distintos caracteres. Desde su punto de vista, la inflación taxonómica del género se vio especialmente incrementada en las últimas décadas del siglo XIX, no sólo por el criterio excesivamente analítico de briólogos como Venturi, Müller y Grönvall, sino también porque no se citaban ejemplares tipo y no se hacían comparaciones exhaustivas en los protólogos (Lewinsky, 1993), lo que provocó una dispersión del conocimiento que se tenía de las especies en distintas áreas geográficas o por distintos especialistas (véase también Britton, 1894).

Asimismo, resulta imprescindible mencionar la relevancia de Dale H. Vitt, quien también realizó una aproximación sintética al género limitándolo a 110 especies (Vitt, 1982), un número muy similar al que alcanzaría Lewinsky en su sinopsis. La labor de Vitt en relación con *Orthotrichum* se ha centrado especialmente en Norteamérica, territorio al que dedicó su principal monografía (Vitt, 1973) y en el que ha continuado su labor desde entonces (Vitt, 2009).

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[2] Lewinsky-Haapasaari a partir de 1994

Las investigaciones de Vitt y Lewinsky también se vieron reflejadas en sendas clasificaciones infragenéricas. Vitt (1971) propuso, a partir de una matriz de datos morfológicos, una división en seis subgéneros en los que resultaban relevantes la conformación del peristoma, el recubrimiento de los estomas, así como la estratificación y papilosidad de los filidios. La sinopsis de Lewinsky (1993) también incluye una división en subgéneros (siete) parcialmente compatible con la de Vitt, y fue discutida años más tarde en el contexto de un análisis cladístico (Lewinsky-Haapasaari y Hedenäs, 1998). Ambas aproximaciones coincidieron en la importancia discriminatoria de la naturaleza de los estomas (superficiales o inmersos). En ambas clasificaciones se coincide también en señalar que la condición de estomas superficiales es plesiomórfica respecto a la de estomas inmersos, y que la adquisición de dichos estomas inmersos tuvo lugar en distintos momentos de la filogenia de forma independiente.

Hasta la fecha no existe una reconstrucción filogenética exhaustiva del género *Orthotrichum* basada en filogenia molecular, aunque sí existen algunas aproximaciones parciales. El principal antecedente es la reconstrucción de las Orthotrichoideae de Goffinet et al. (2004), que presenta un panorama diferente al de las divisiones infragenéricas llevadas a cabo por Vitt y Lewinsky. En primer lugar, el género *Orthotrichum* no constituiría un grupo monofilético, sino que estaría segregado principalmente en dos clados: el basal incluiría las especies de estomas superficiales y estaría estrechamente ligado al género *Ulota* Mohr, mientras que en el segundo se englobarían las especies de estomas inmersos, así como los antiguos géneros *Muelleriella* Dusén y *Orthomitrium* Lewinsky-Haapasaari & Crosby. Además, en una posición intermedia entre ambos clados, se situarían los géneros *Stoneobryum* Norris & Robinson y *Sehnemobryum* Lewinsky-Haapasaari & Hedenäs. Finalmente, quedarían excluidos de *Orthotrichum* los géneros *Nyholmiella* Holmen & Warncke y *Leratia* Brotherus, cuyas especies tradicionalmente se habían considerado integrantes del mismo. Aunque la representación de especies de *Orthotrichum* de estomas inmersos no es muy extensa en este estudio, sus resultados serían compatibles con la adquisición de los estomas inmersos en una única ocasión.

Varios trabajos recientes (Sawicki et al., 2009a; 2009b; 2010; 2012) han acometido aproximaciones parciales a la filogenia del género *Orthotrichum* y a la revisión de su clasificación infraespecífica. En líneas generales coinciden con lo expuesto anteriormente con respecto al género *Nyholmiella* y a la posición de las especies de estomas superficiales e inmersos en la filogenia, aunque más allá de eso no parece haber una compatibilidad con las clasificaciones propuestas anteriormente por Vitt y Lewinsky, excepto quizá para el caso los taxones incluidos en el subgénero *Orthotrichum*. Los resultados de estos estudios señalan, como en el caso anterior, que la adquisición de estomas inmersos tuvo lugar una única vez en la filogenia.

Justificación del estudio y estructura de la memoria

De lo expuesto anteriormente se desprende que, a pesar de haber recibido una atención considerable a lo largo de los últimos siglos, la diversidad específica del género *Orthotrichum* aún dista de encontrarse completamente conocida. A las complicaciones características de los briófitos con relación a la delimitación de especies habría que añadir las propias de un género que ha supuesto a menudo un desafío a los briólogos por su complejidad intrínseca.

En esta memoria se abordan una serie de cuestiones taxonómicas relacionadas con la delimitación de especies del género *Orthotrichum*, subgénero *Pulchella* (sensu Lewinsky, 1993) en el hemisferio norte. En ellas son recurrentes varios de los aspectos mencionados anteriormente: 1) la dificultad taxonómica intrínseca de unos organismos morfológicamente sencillos pero muy variables, 2) la premisa de la existencia de distribuciones muy amplias, incluyendo disyunciones transoceánicas, que sin embargo no se han re-evaluado de forma crítica y 3) la ausencia, hasta la fecha, de estudios moleculares en este género que tengan como objetivo alcanzar soluciones taxonómicas de carácter integrativo.

Estos estudios se presentan en el capítulo IV en forma de artículos científicos, publicados o aún inéditos, ordenados cronológicamente, a los que se mencionará según su numeración en la presente memoria. Se puede encontrar en cada uno de ellos una introducción detallada sobre sus antecedentes, por los que aquí nos limitamos a presentarlos de forma resumida.

- En primer lugar, se aborda el caso de *Orthotrichum scanicum* Grönvall, una especie europea incluida en la Lista Roja Mundial de Briófitos Amenazados por considerarse muy localizada y escasa. Durante los últimos años, el incremento en el número de citas a lo largo del continente y por el resto de la Región Mediterránea y Oriente Medio nos sugiere que su conocimiento era en realidad insuficiente y que es necesaria una delimitación morfológica más precisa (capítulo IV.2). Además, vemos pertinente estudiar sus posibles afinidades con la especie asiática *O. holmenii* Lewinsky-Haapasaari (IV.2) y con una serie de especímenes procedentes de California identificados originalmente como *O. tenellum* Bruch ex Brid. (IV.1), con los que mantienen semejanza. En este estudio no se realizan estudios moleculares, pero se incluyen materiales de *O. scanicum* en IV.4 y IV.5.

- El segundo estudio está centrado en la re-evaluación de *Orthotrichum consimile* Mitt., una especie con una compleja historia taxonómica (cuenta con más de una docena de sinónimos) que se considera distribuida por la fachada pacífica de Norteamérica y las regiones oceánicas de Europa. Una notable variabilidad morfológica en los especímenes americanos hace pensar en que la delimitación que se estaba asumiendo no refleja la diversidad real de un posible complejo de especies crípticas. Por este motivo se lleva a cabo un estudio que comprende tanto una profunda revisión morfológica como una reconstrucción filogenética (capítulo IV.4).

- Finalmente, se aborda el caso de *Orthotrichum tenellum* Bruch ex Brid. Al igual que en el estudio anterior, se parte de su consideración como especie disyunta, presente en esta ocasión en California y la Región Mediterránea, así como en varios territorios adyacentes. Una vez más, comprobamos que se trata de un taxón con una historia muy compleja en el que se detecta una variabilidad morfológica no congruente con la delimitación actual. Los capítulos IV.1 y IV.3 inician el estudio de dos de las formas americanas que se han estudiado, culminando con el estudio integrativo de IV.5.


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II.

Objetivos

"Aunque se piense que esto es también una empresa interminable y que no ha de tratarse a la ligera sin ser objeto de una cierta crítica, en ninguna clase de asuntos es más justa la indulgencia, salvo que se tenga por extraño que uno que ha nacido hombre no posea todos los conocimientos humanos"

Plinio el Viejo, ca. 77
Historia Natural III.1

Conforme a lo expresado en las líneas anteriores, y con el objetivo general de contribuir a un mejor conocimiento de la taxonomía, la filogenia y la biogeografía del género *Orthotrichum*, esta tesis se ejecutó con los siguientes objetivos fundamentales:

1. Delimitar taxonómicamente la especie *Orthotrichum scanicum* en relación con sus afines, actualizar su distribución y aportar datos para la valoración de su estado de conservación.

2. Resolver y describir la diversidad subyacente en *Orthotrichum consimile* integrando una revisión morfológica y datos filogenéticos que permitan determinar si se trata de un complejo de especies crípticas.

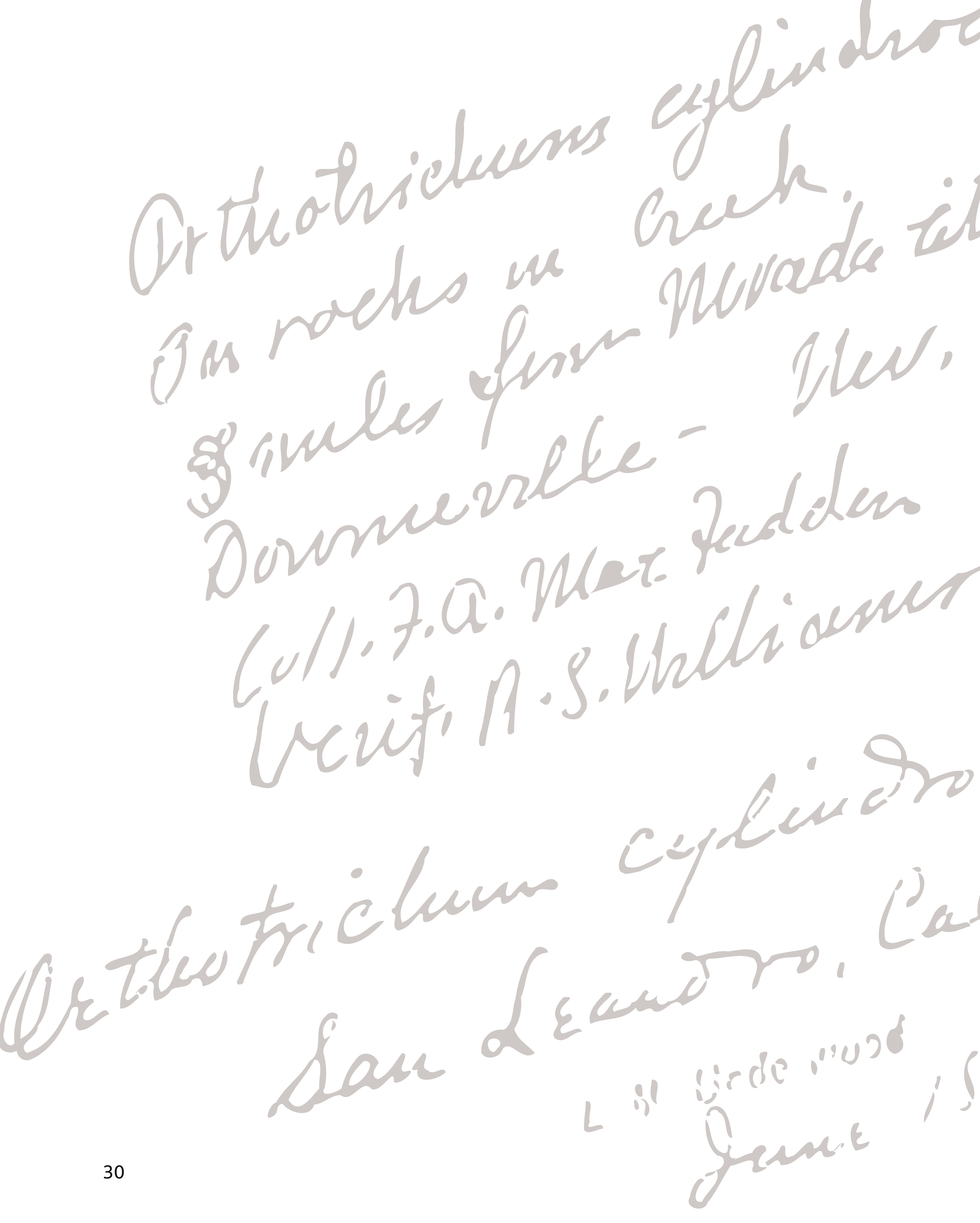
3. Resolver y describir, mediante la taxonomía integrativa, la diversidad adscrita a *Orthotrichum tenellum* tanto en la Región Mediterránea como en la Región florística de California y áreas adyacentes.

De forma complementaria, se han abordado otros objetivos necesarios para la consecución de los propósitos de la investigación:

4. Identificar y evaluar los caracteres morfológicos que presenten utilidad taxonómica en el marco de las especies estudiadas.

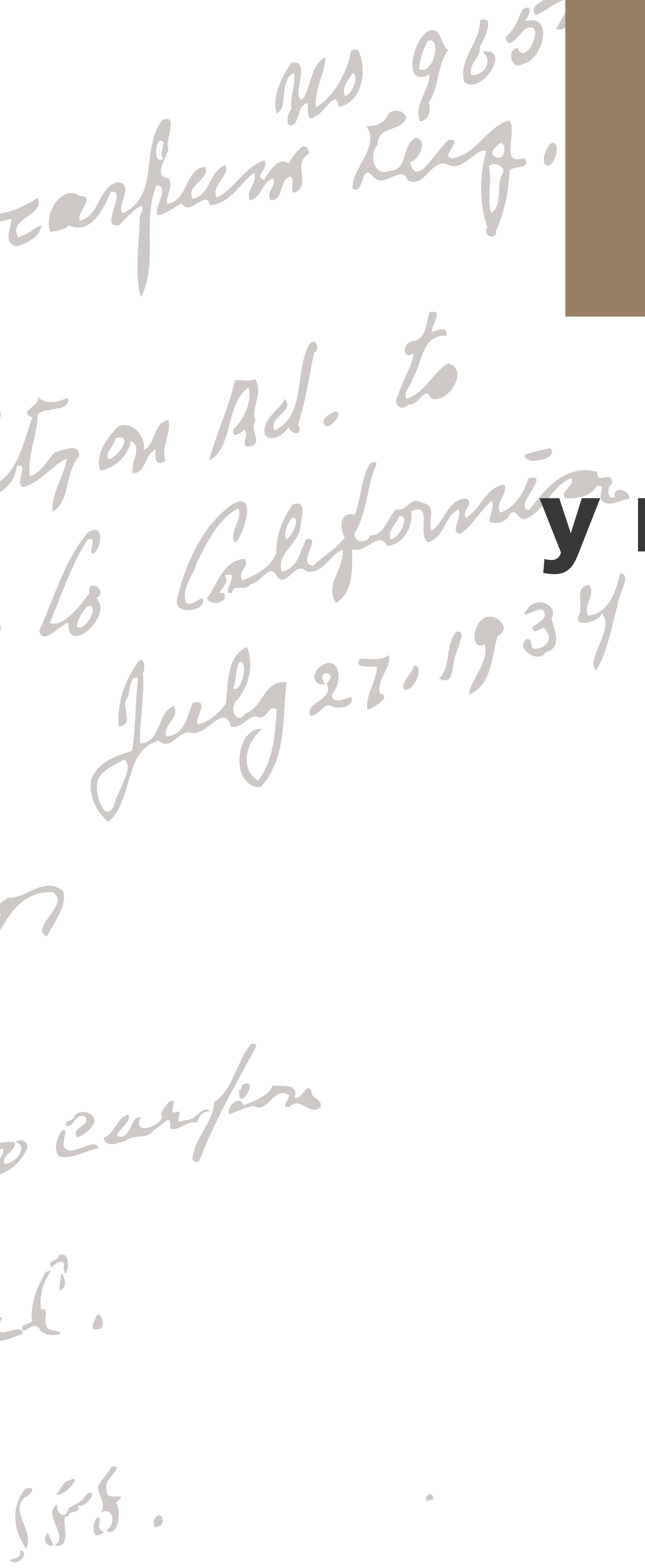
5. Encontrar regiones del genoma que tengan utilidad para el estudio filogenético de los taxones considerados en la memoria así como para el resto del subgénero *Pulchella*.

6. Aportar datos para la reconstrucción filogenética de las especies del subgénero *Pulchella*, y para mejorar el conocimiento de su biogeografía.



Orthotrichum cylindroc
On rocks in Creek.
8 miles from Nevada Cit
Downieville - Nev,
Coll. F.A. MacFadden
Verif. R.S. Williams

Orthotrichum cylindro
San Leandro, Ca
L. M. Underwood
June 1 S



III.

Material y métodos

"Taxonomy can thus
be a dangerous field to work in."
Jette Lewinsky, 1993
A synopsis of the genus *Orthotrichum*

Análisis morfológico

La revisión taxonómica del presente estudio incluye el examen de más de 750 especímenes recolectados principalmente en dos áreas geográficas. La primera abarca la región pacífica de Norteamérica, desde la Columbia Británica hasta México y muy especialmente el estado de California, si bien se incluyen territorios más interiores como el estado de Nevada. En segundo lugar se han estudiado materiales de Europa y la Región Mediterránea, incluyéndose además especímenes de las islas Canarias, Turquía, Georgia y Kazajistán.

Una parte de estos ejemplares proceden de los herbarios ALTA, B, CAS, COLO, FH, MICH, MO, NY, SFSU, TR y UC, además del herbario del laboratorio de Briología de la Universidad Autónoma de Madrid. Los ejemplares americanos de recolección más reciente proceden en gran parte de las campañas de muestreo llevadas a cabo en 2008 y 2011 por Francisco Lara, Ricardo Garilleti y Belén Albertos. También se han utilizado especímenes recolectados por diversos briólogos: Benjamin Carter, Colin Dillingham, Belén Estébanez, Bernard Goffinet, Vicente Mazimpaka, Nagore G. Medina, Daniel H. Norris, James R. Shevock y David Toren, así como por el autor de esta memoria.

A continuación se enumeran los caracteres morfológicos examinados en cada espécimen en esta parte del estudio y que aparecen en las descripciones (véase también figura III.1). Se comenta además la variación de estos caracteres en el contexto del género *Orthotrichum* (s.l.) tomando como base la revisión de Lewinsky (1993) y la experiencia acumulada por el Equipo de la Universidad Autónoma de Madrid y Universidad de Valencia durante cerca de veinte años de investigaciones en este género.

Caracteres taxonómicos

Colonias y hábito. Se examinaron el tamaño de las colonias, su forma de crecimiento, color y longitud de los gametóforos. Las especies del género *Orthotrichum* crecen en almohadillas, densas o laxas. Las plantas más pequeñas miden alrededor de tan solo 1 mm de longitud, mientras que en el extremo contrario hay especies que alcanzan los 5 o 6 cm. El color de las mismas suele ser, habitualmente, verde claro o verde oliva, pero puede variar desde casi negro hasta verde claro, pasando por tonalidades pardas y amarillentas, ocasionalmente incluso glaucas.

Rizoides. Se ha examinado principalmente su carácter ascendente a lo largo del caulidio, su color y su superficie (lisa o rugosa). En *Orthotrichum* suelen estar limitados

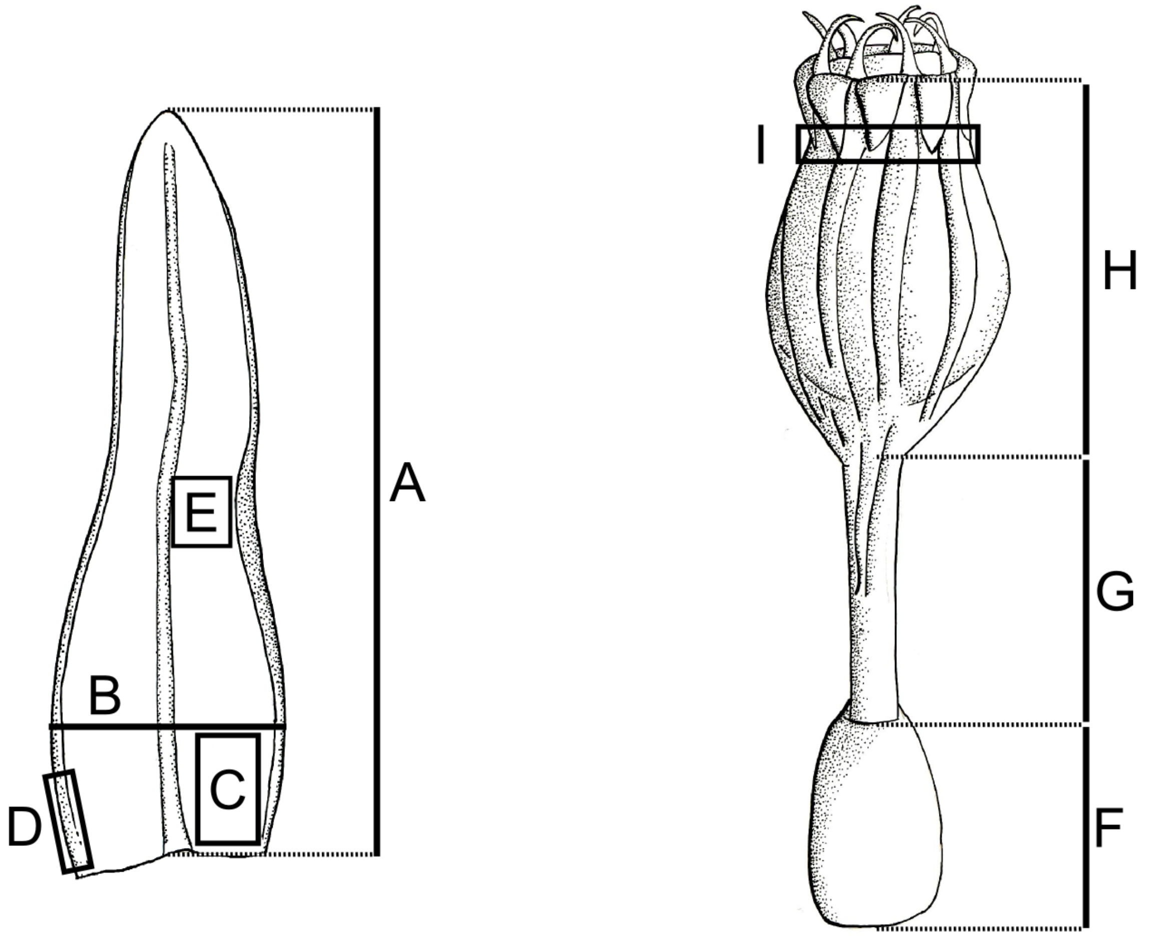


FIG. III.1. Algunas de las medidas empleadas para el estudio morfológico y zonas donde se han efectuado. A. Longitud del filidio. B. Anchura del filidio. C. Área de medida de las células basales. D. Área de medida de las células basales marginales. E. Área de medida de las células del centro de la lámina. F. Longitud de la vagínula. G. Longitud de la seta. H. Longitud de la cápsula. I. Área de observación y medida de las células y bandas exoteciales. (Dibujos por R. Caparrós).

a las regiones más basales del gametóforo, aunque pueden ascender en mayor o menos medida. Típicamente son marrones o rojizos.

Caulidios. Fundamentalmente se describe la forma de su sección transversal, que varía desde subpentagonal a más o menos redondeada, y el grosor de la esclerodermis, constituida por un número variable de células pequeñas con paredes engrosadas.

Pelos axilares. En su descripción se atiende tanto a su forma como a su longitud y número de células que los componen. Por norma se trata de estructuras filiformes o subclaviformes formadas por células alargadas e hialinas, con la excepción de una o dos células basales coloreadas.

Filidios. Los filidios constituyen el órgano del gametofito más versátil y rico en caracteres morfológicos con utilidad taxonómica para este género. Durante la revisión se prestó atención a su disposición tanto en seco como en húmedo, a su tamaño (longitud y anchura máxima) a la forma y areolación de la lámina y del ápice foliar, así como a la estratificación de la lámina, morfología del margen y del nervio.

La disposición de los filidios en seco puede ser desde adpresa a contorta, incluyendo todos los grados intermedios. Cuando se hidratan se disponen entre erectos y patentes respecto del eje del caulidio. En cuanto al tamaño, en las especies más robustas, los filidios pueden llegar a los 5-6 mm de longitud, siendo lo más habitual que ésta permanezca entre los 3-4 mm. Además, el tamaño de los mismos varía normalmente a lo largo del caulidio, a veces de manera muy marcada, haciéndose más grandes hacia el extremo del gametóforo. El espectro de formas comprende filidios lineares, lanceolados, oblongos y ovados.

Se han prestado además una atención especial a los ápices de los filidios y su variabilidad. Generalmente son ápices sencillos que varían en su ángulo, de acuminados a redondeados. Sin embargo, especializaciones tales como aristas, canales, apículos o mucrones son muy frecuentes, aunque ha de tenerse en cuenta que la variabilidad de los ápices es grande tanto dentro del mismo taxón como, muy frecuentemente, en un mismo individuo.

Se ha descrito además la estratificación de la lámina y la morfología del margen del filidio. En el género *Orthotrichum* lo habitual es que la lámina sea unistrata pero las láminas parcial o totalmente bistratas también se dan de forma excepcional. El margen del filidio suele presentarse variablemente recurvado, o más raramente, plano e incluso incurvo en algunos taxones. En cuanto al nervio, se incluyen las medidas de su anchura en la base y en el centro de la lámina, así como la descripción de su sección transversal. El nervio posee dos células guía ventrales y por norma general no llega a alcanzar el ápice del filidio.

Finalmente, se han descrito la forma y tamaño de las células basales del filidio, las células basales marginales y las células del centro de la lámina. Las células basales son elongadas, hialinas (raramente coloreadas) y con paredes de grosor variable y hasta nodosas en algunos casos. Las células basales marginales son subcuadradas y con paredes levemente engrosadas. Hacia el centro de la lámina, las células son isodiamétricas o algo elongadas, con paredes de grosor variable. La superficie de las células del centro de la lámina puede ser lisa, aunque generalmente aparecen sobre ella papilas de distinto desarrollo.

Yemas. Se ha examinado la presencia de propágulos de reproducción vegetativa y los órganos sobre los que se desarrollan. En su caso, se ha analizado la forma, el tamaño y el número de células que componen estas yemas. Habitualmente están constituidas por 4-7 células y son vermiformes o fusiformes, si bien existen en el género especies con yemas más largas y ramificadas. Suelen formarse en la lámina de los filidios, aunque ocasionalmente pueden observarse creciendo a partir de los extremos de algunos rizoides.

Sexualidad. En este epígrafe se ha descrito la distribución de los gametangios en el gametofito y, someramente, los filidios de los mismos en el caso de que presenten alguna diferenciación respecto a los vegetativos.

La condición autoica es, con diferencia, la más extendida en el género *Orthotrichum* (s.l.), que comprende taxones tanto cladautoicos como goniautoicos. Únicamente son dioicos *O. lyellii* y las especies que en la actualidad están incluidas en el género *Nyholmiella*. Los filidios periqueciales rara vez están diferenciados respecto a los vegetativos, aunque sí es frecuente que sean más grandes. Los filidios perigoniales, por el contrario, son de tamaño reducido, generalmente ovados, con el nervio muy corto y células típicamente romboidales.

Vagínula. Se ha examinado tanto su longitud como su forma, que generalmente es cilíndrica, en ocasiones truncada o doliforme. La presencia de pelos en este órgano, su longitud y constitución también se han examinado detenidamente. En caso de estar presentes, su densidad, longitud y superficie (grosor de la pared celular y papilosidad) puede ser de relevancia taxonómica.

Caliptra. En relación con este órgano, han sido objeto de estudio su forma a lo largo de su desarrollo y, en el caso de presentar pelos, la constitución y ubicación de los mismos. En el género *Orthotrichum* las caliptras son típicamente mitradas y estriadas longitudinalmente, con una forma que va desde oblonga a cónica o incluso fusiforme y, muy a menudo, acaban en un rostro que puede ser de color más oscuro. Al igual que en el caso de la vagínula, los pelos, si están presentes, son variables en longitud, areolación y superficie.

Seta. Se ha examinado fundamentalmente su longitud y torsión. En el género *Orthotrichum* la seta puede variar sustancialmente en longitud, desde 0.2 mm hasta más de 5 mm. La seta presenta una torsión antihoraria, excepto cuando es muy corta, en cuyo caso no suele apreciarse torsión alguna. En algunas especies la seta puede doblarse levemente hacia abajo. La relación entre la longitud de la seta la de las hojas periqueciales es responsable del grado de elevación de la cápsula que puede ser inmersa, emergente o exerta.

Cápsula. La forma de la cápsula varía a lo largo de su desarrollo, por lo que en las descripciones aparece tanto su forma juvenil como la que adquiere en la madurez. En el conjunto del género se encuentran desde cápsulas globosas a largamente cilíndricas, variablemente constreñidas bajo la boca, con superficie lisa o variablemente estriada y con el cuello defluente o abruptamente contraído.

Opérculo. Se ha prestado atención especialmente a su forma, que puede ser plana, cónica o convexa. Es habitual la presencia de un anillo basal oscuro, aunque su ausencia es característica de algunas especies. Siempre presenta un rostro de longitud variable.

Exotecio. Se han estudiado y medido las células exoteciales normales así como las células diferenciadas que constituyen las bandas longitudinales (costillas) de la urna, cuando estaban presentes. Típicamente las células exoteciales normales son elongadas y casi hialinas, mientras que las que constituyen las bandas exoteciales son rectangulares, con paredes engrosadas y coloreadas. La anchura de estas bandas (expresada en función del número de filas de células que las forman) también se ha examinado en este trabajo.

Estomas. Los caracteres relevantes que se observaron en estas estructuras fueron su posición en la urna y su naturaleza superficial o inmersa (y en este último caso, el grado de recubrimiento por parte de las células adyacentes).

Peristoma. El peristoma es una estructura que alberga un buen número de caracteres de relevancia taxonómica. En primer lugar se ha observado su constitución, es decir, el número de piezas que forman el exostoma y el endostoma y su posición relativa. La constitución básica en este género sigue un esquema artrodonto diplolépido con exostoma y endostoma de dieciséis piezas dispuestas de manera alterna. Esta situación original se encuentra, la mayor parte de las veces, modificada por la fusión o la pérdida de algunas de las piezas que lo integran. Así, el exostoma muestra con mucha frecuencia ocho estructuras (pares de dientes) que son el resultado de la unión dos a dos de dientes adyacentes del juego original. Estas parejas pueden presentar o no una tendencia a la escisión cuando el esporofito madura. En cuanto al endostoma, es muy frecuente que a partir de los dieciséis segmentos originales se produzca la reducción o la pérdida de las ocho piezas intermedias (las que se enfrentan con la línea media del par de dientes fusionado del exostoma).

También se ha examinado la forma y longitud tanto de los dientes del exostoma como de los segmentos del endostoma. Los dientes (o pares de dientes) del exostoma presentan un contorno triangular cuando están bien desarrollados, en ocasiones con el ápice truncado o erosionado. Por su parte los segmentos tienen una forma más variable: generalmente lineares con la base más o menos ensanchada en ocasiones apendiculados o lanceolados e incluso fusionados entre sí. Además se estudiaron las superficies (interna y externa) de dichos dientes y segmentos, que pueden ser lisas o presentar ornamentaciones muy variadas (papilas, líneas, estrías, rugosidades, etc.), tanto de manera uniforme como mostrando diferencias entre la base y el ápice del diente o segmento.

Esporas. Se han medido sus diámetros y descrito la ornamentación de su superficie. Las esporas en *Orthotrichum* son subesféricas y papilosas, casi siempre unicelulares. Generalmente su diámetro suele oscilar entre 12 y 25 µm, aunque algunas especies de estomas superficiales presentan esporas mucho mayores.

Filogenia molecular

El proceso de extracción, amplificación, purificación y secuenciación de material genético y su posterior análisis se realizó en distintos centros de investigación: la Universidad de Murcia (Departamento de Biología Vegetal), la Universidad de Connecticut (Departamento de Ecología y Biología Evolutiva) y la Universidad Autónoma de Madrid (Departamento de Biología). Al tratarse de un género en el que todavía no se habían realizado muchos estudios filogenéticos, el trabajo conllevó una carga importante de puesta a punto de protocolos, selección de marcadores moleculares y diseño de cebadores específicos.

En la medida de lo posible, para la extracción de ADN se seleccionó, de entre los materiales de más reciente recolección, una muestra representativa tanto de la variabilidad morfológica como de la amplitud geográfica de las especies estudiadas, añadiendo además especímenes selectos del contexto filogenético (subgéneros *Pulchella* y *Orthotrichum*) y un grupo externo compuesto por ejemplares de *Ulota crispa*, *Nyholmiella obtusifolia* y especies de *Orthotrichum* de estomas superficiales.

Extracción de material genético

La extracción de ADN se ha realizado por el protocolo de digestión con NaOH (Werner et al., 2002) o mediante el método de extracción por hexadeciltrimetilamonio, CTAB (Doyle, 1987) modificado para su uso con ortotricáceas según se describe en Goffinet et al. (1998). Alternativamente se empleó el kit comercial DNeasy® Plant Mini Kit (Qiagen) siguiendo las instrucciones del fabricante. El método de extracción no ha constituido un factor determinante para el trabajo posterior, aunque sí se ha puesto de manifiesto, independientemente del protocolo empleado, una dificultad frecuente en obtener extractos útiles a partir de materiales de herbario de más de 8-10 años desde la fecha de recolección.

Selección de regiones genómicas

Una parte significativa del trabajo de laboratorio consistió en la búsqueda de regiones adecuadas para la reconstrucción filogenética en los tres genomas. Finalmente, se emplearon dos conjuntos de secuencias: cuatro regiones del genoma cloroplástico y dos del genoma nuclear.

Los marcadores estudiados pertenecientes al genoma cloroplástico son *atpB-rbcL, rps4, trnG* y *trnL-F*, todos ellos empleados con anterioridad en otros estudios sistemáticos de briófitos (Stech & Quandt, 2010). Los pares de cebadores usados fueron *atb1/ rbcL1* (Chiang et al., 1998); *rpsA/trnaS* (Nadot et al., 1994); (Souza-Chies et al., 1997); trnGF/trnGR (Pacak & Szweykowska-Kulinska, 2000) y *trnC/trnF* (Taberlet et al., 1991) respectivamente.

Por otro lado, del genoma nuclear se seleccionaron dos regiones: *AdK* y *ort-Lfy*. En ambos casos se consiguió la amplificación de las regiones mediante una PCR anidada, por lo que además de los cebadores externos *AdK-F/AdKR* (McDaniel et al., 2010) y *LFY1428F/LFY2327R* (Shaw et al., 2003a) se diseñaron unos cebadores internos específicamente para los especímenes de *Orthotrichum*: *ort-AdKF/ort-AdKR* y *ort-LfyF/ort-LfyR* (tabla IV.5.1). Posteriormente se ha comprobado que la región *ort-Lfy* es en realidad una región anónima que no se corresponde con ninguna de las secuencias existentes en GenBank hasta la fecha.

De entre las regiones cloroplásticas ensayadas pero desestimadas para los análisis moleculares, se pueden mencionar *trnC-RpoB*, *trnV-r* y *trncF-rpoBR2*. La desestimación de estas regiones se debió tanto a la observación de una variabilidad limitada en el seno del subgénero *Pulchella* como a dificultades en la amplificación. Por su parte, también se desestimaron varias regiones del genoma nuclear: *ITS1, ITS2, RPO*, intrones de *GapC* y *Leafy-Physco*. Las regiones *ITS* han demostrado en otras ocasiones su utilidad en las plantas verdes para estudios de filogenia a nivel interespecífico (Stech & Quandt, 2010) y recientemente se han empleado en el género *Orthotrichum* s.l. (p. ej. Sawicki et al., 2009a). Sin embargo, en el transcurso de los estudios realizados en el marco de esta tesis doctoral, dichas regiones presentaron serios problemas de amplificación y replicabilidad, además de producir con frecuencia bandas dobles y varios juegos de secuencias que hacen pensar en la presencia de secuencias parálogas en distintas Regiones Organizadoras Nucleolares. Esta circunstancia coincidiría con estudios inéditos realizados con anterioridad en *Orthotrichum lyellii* (Goffinet, com. pers.).

Por último, se ensayó también la amplificación de la región *trnC* del genoma mitocondrial, que fue desestimada por la ausencia de variabilidad entre secuencias. Esto es algo habitual en el genoma mitocondrial vegetal, que por lo general está relativamente bien conservado (Shaw, 2008).

Amplificación por PCR

En su versión optimizada por el proceso de experimentación, la amplificación por PCR de las regiones seleccionadas se hizo en volúmenes finales de 25 µL con 0.2 µL de Taq polimerasa (Taq Polymerase / HotMaster Taq Polymerase 5PRIME), 2.5 µL del tampón con Mg^{2+} suministrado por el comerciante, 1 µL de una mezcla de dNTPs 10 µM, 1 µL de la alícuota de cada cebador (10 µM) y 1 µL del extracto de ADN. En el caso de las PCRs anidadas, para la reacción interna se empleó 0.5 µL del producto de la reacción externa. Tras una desnaturalización a 94 ºC durante 5 minutos se iniciaban 30 ciclos consistentes en una desnaturalización de 30 segundos a 94 ºC, una hibridación de 1 minuto a la temperatura correspondiente para cada región (tabla IV.5.1) y una extensión de 30 segundos a 68 ºC. La reacción concluía después de una fase de extensión adicional de 7 minutos a 68 ºC.

Los productos de PCR fueron visualizados en geles al 1% de agarosa y purificados usando el kit comercial Nucleospin Extraction Kit (Machery-Nagel). Las reacciones de secuenciación se realizaron en volúmenes finales de 10µL con la enzima ABI BigDye Terminator y purificadas como se explica en (Buck et al., 2000) antes de proceder a la lectura de las secuencias en un ABI Prism 3100 Genetic Analyser. En los casos en los que el laboratorio donde se realizaron las amplificaciones no contaba con los medios necesarios, las muestras se enviaron a secuenciar en otro centro. Los cebadores empleados en la secuenciación fueron los mismos que los que se usaron en la reacción de PCR.

Análisis filogenético

Las lecturas parciales para cada cebador fueron ensambladas y corregidas usando Sequencher 3.1.1. (Machery-Nagel) y Geneious 5.1.7.0 (Biomatters Ltd.). Las matrices para cada región se construyeron con PhyDE (Müller et al., 2006). Las secuencias de cada matriz se alinearon mediante los programas ClustalW (Larkin et al., 2007) y MUSCLE (Edgar, 2004) y fueron revisadas manualmente después. Las matrices parciales se ensamblaron en matrices compuestas: una para las cuatro regiones cloroplásticas y otra para las dos regiones nucleares, pero, respetando su diferente origen, se realizaron particiones para cada marcador y se comprobó su compatibilidad mediante el test ILD (Farris et al., 1994). En el caso de que se detectase incongruencia entre pares de marcadores, se comprobó examinando sus resultados por separado que ésta no afectaba al nivel de la delimitación específica.

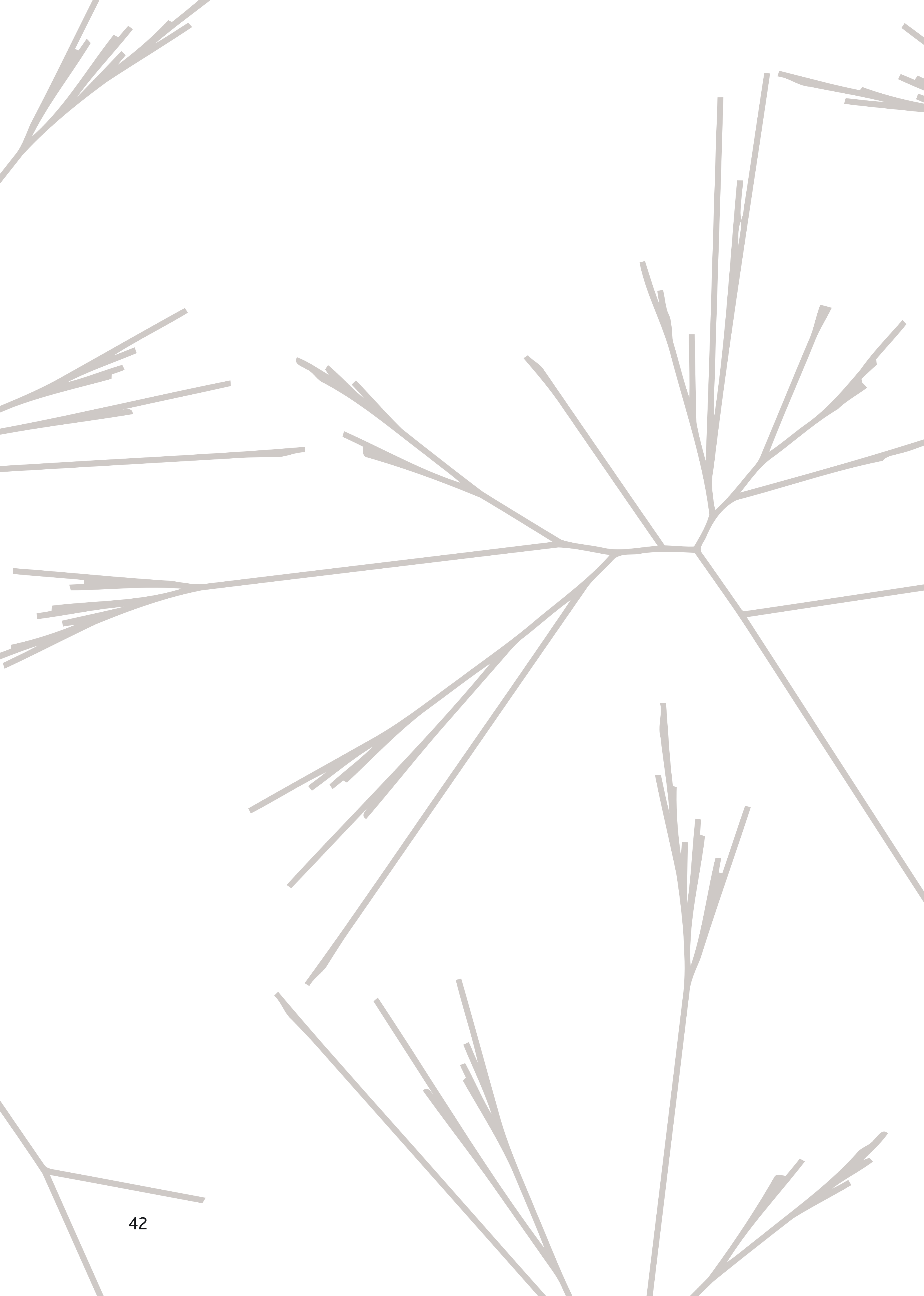
El modelo de sustitución nucleotídica más plausible se estimó para cada uno de los marcadores, tanto del genoma cloroplástico como del nuclear, usando jModelTest 0.1.1 (Posada, 2008) e implementándolos, como se ha dicho, de forma independiente en cada partición. A cada una de las matrices compuestas se le añadió un bloque que contenía las indels codificadas de toda la matriz, de acuerdo con la codificación simple que aparece en Simmons & Ochoterena (2000), tal y como está implementado en SeqState 1.4 (Muller, 2005). El modelo de sustitución aplicado para el bloque de indels fue el F81 según se recomienda en Ronquist et al. (2005).

Se realizaron análisis de tres tipos: Máxima Parsimonia (MP), Máxima Verosimilitud (MV) e Inferencia Bayesiana (IB). Los análisis de MP se realizaron mediante el programa MEGA5 (Tamura et al., 2011) aplicando 1000 pseudorréplicas de bootstrap (Felsenstein, 1985) como estimador de la robustez de los clados en los árboles resultantes.

Los análisis de MV se realizaron con Garli 2.0 (Zwickl, 2006), que permite la estimación de la verosimilitud respetando las particiones de la matriz. El programa se ejecutó empleando 200 pseudorréplicas de bootstrap como método para evaluar la robustez de los clados. El árbol consenso se obtuvo mediante el programa SumTrees implementado en DendroPy (Sukumaran & Holder, 2010).

Los análisis de IB se llevaron a cabo con MrBayes 3.1.2. (Ronquist & Huelsenbeck, 2003) respetando el modelo de sustitución para cada una de las particiones. El número de generaciones de cada análisis dependía del tiempo empleado en alcanzar la convergencia de cada una de las cadenas MCMC, variando entre 2 y 5 millones. En los capítulos correspondientes se exponen con detalles los parámetros particulares bajo los cuales se realizaron cada uno de los análisis filogenéticos. Los resultados se visualizaron con el editor gráfico de MEGA5 y FigTree 1.3.1. (Rambaut & Drummond, 2010) y fueron combinados y editados después manualmente.

Para complementar los resultados filogenéticos, se empleó el gsi_T (Genealogical Sorting Index; Cummings et al., 2008) como un test de monofilia para los grupos de especímenes pertenecientes a las especies cuya delimitación se pretendía comprobar. El gsi_T es un estadístico que cuantifica el grado de monofilia exclusiva de grupos delimitados manualmente, frente a la hipótesis nula de que se trata de agrupaciones estocásticas.



IV.

Resultados

"After describing a set of forms as distinct species, tearing up my manuscript and making them one species, tearing that up and making them separate, and then making them one again (which has happened to me) I have gnashed my teeth, cursed species and asked what sin I had committed to be so punished"

Carta de Charles Darwin a Joseph D. Hooker,
25 de septiembre de 1853

IV.1

Orthotrichum norrisii

IV.1 *Orthotrichum norrisii* (Orthotrichaceae), a new epiphytic Californian moss

IV.1 *Orthotrichum norrisii* (Orthotrichaceae), un nuevo musgo epífito de California

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Abstract

A new *Orthotrichum* species, *O. norrisii*, is described from California. The new taxon is recognized by a unique combination of sporophytic characters: capsule narrowly cylindrical and not contracted below mouth when dry, exothecial bands narrow and formed by 2(–3) cell rows, peristome with eight pairs of exostome teeth and eight endostome segments, stomata cryptopore and located in the lower part of the urn, lid plane and rostrate and vaginula hairy. Widely distributed throughout California, the new taxon has been overlooked and confused with *O. tenellum*.

Resumen

Se describe una nueva especie de *Orthotrichum*, *O. norrisii*, de California. El nuevo taxón es reconocible por una combinación única de caracteres: cápsula estrechamente cilíndrica y no contraída bajo la boca en seco, bandas exoteciales estrechas, formadas por 2(-3) filas de células, peristoma compuesto por ocho pares de dientes en el exostoma y ocho segmentos en el endostoma, estomas criptoporos situados en la parte baja de la urna, opérculo plano y rostrado y vagínula pelosa. Distribuido ampliamente por toda California, el nuevo taxón ha pasado desapercibido anteriormente y se ha confundido con *O. tenellum*.

Introduction

Orthotrichum is one of the most diverse moss genera, and it is represented on all continents in a wide range of habitats, with a special prominence in epiphytic communities of temperate forests (Lewinsky 1993). The taxonomy of this genus has experienced significant advances during the last decades (Lewinsky 1993; Vitt 1973), but several problems still persist. Subgenus *Pulchella* (Schimp.) Vitt *sensu* Lewinsky (1993) is especially diverse and problematic, since this group of small-statured epiphytic mosses includes several taxonomic complexes not yet formally defined, in which important difficulties for species delimitation exist. One of these complexes comprises the species related to *Orthotrichum tenellum* Bruch ex Brid., which is one of the current objects of study of our research team.

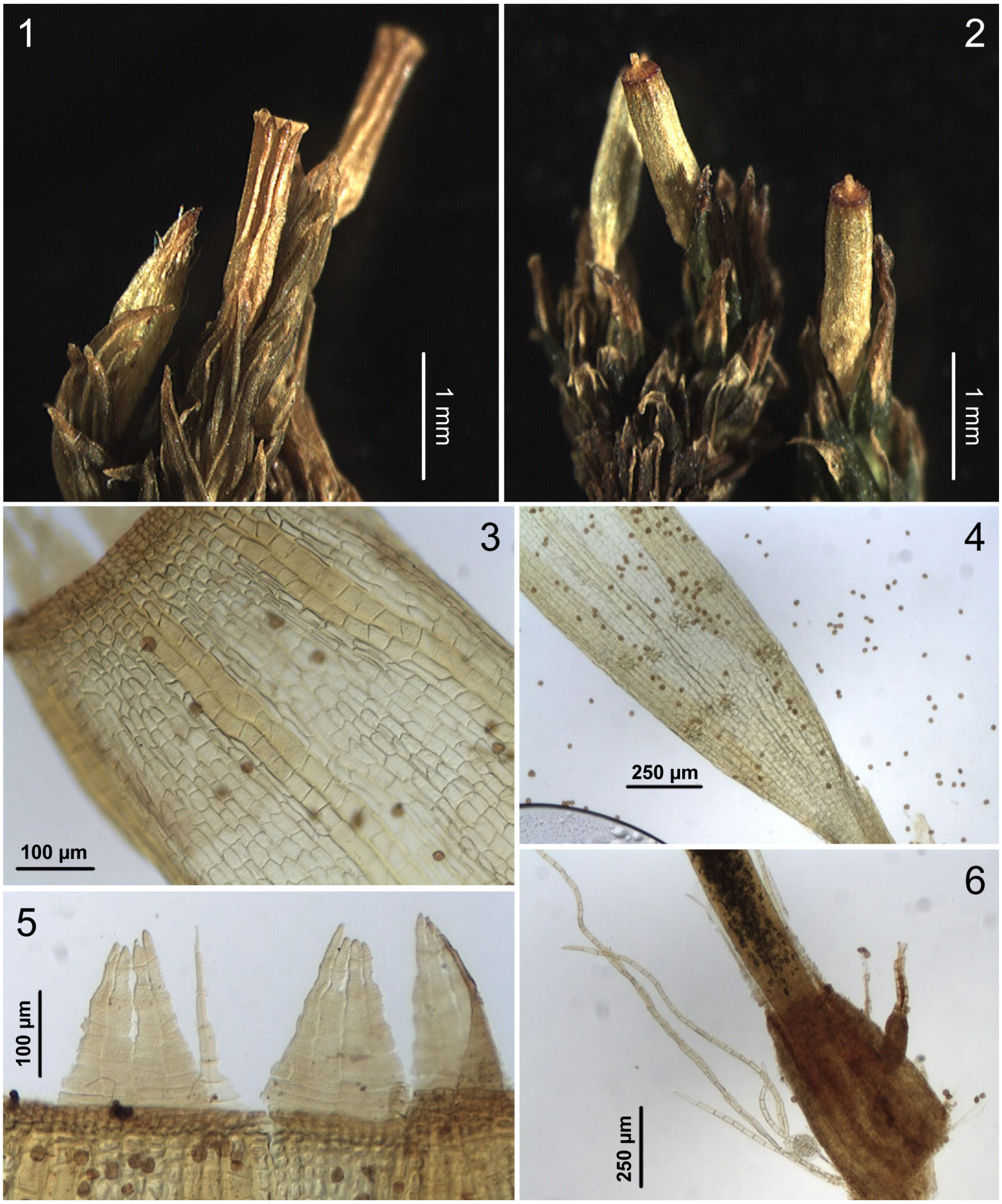
While revising materials of North American *Orthotrichum tenellum*, several Californian specimens caught our attention by their characteristic sporophytes with narrow exothecial bands, remarkably different from those of *O. tenellum*. A more detailed study showed other differential sporophytic characters that support the recognition of a new species.

Species description

Orthotrichum norrisii F. Lara, R. Medina & Garilleti, sp. nov. Figs. IV.1.1–12

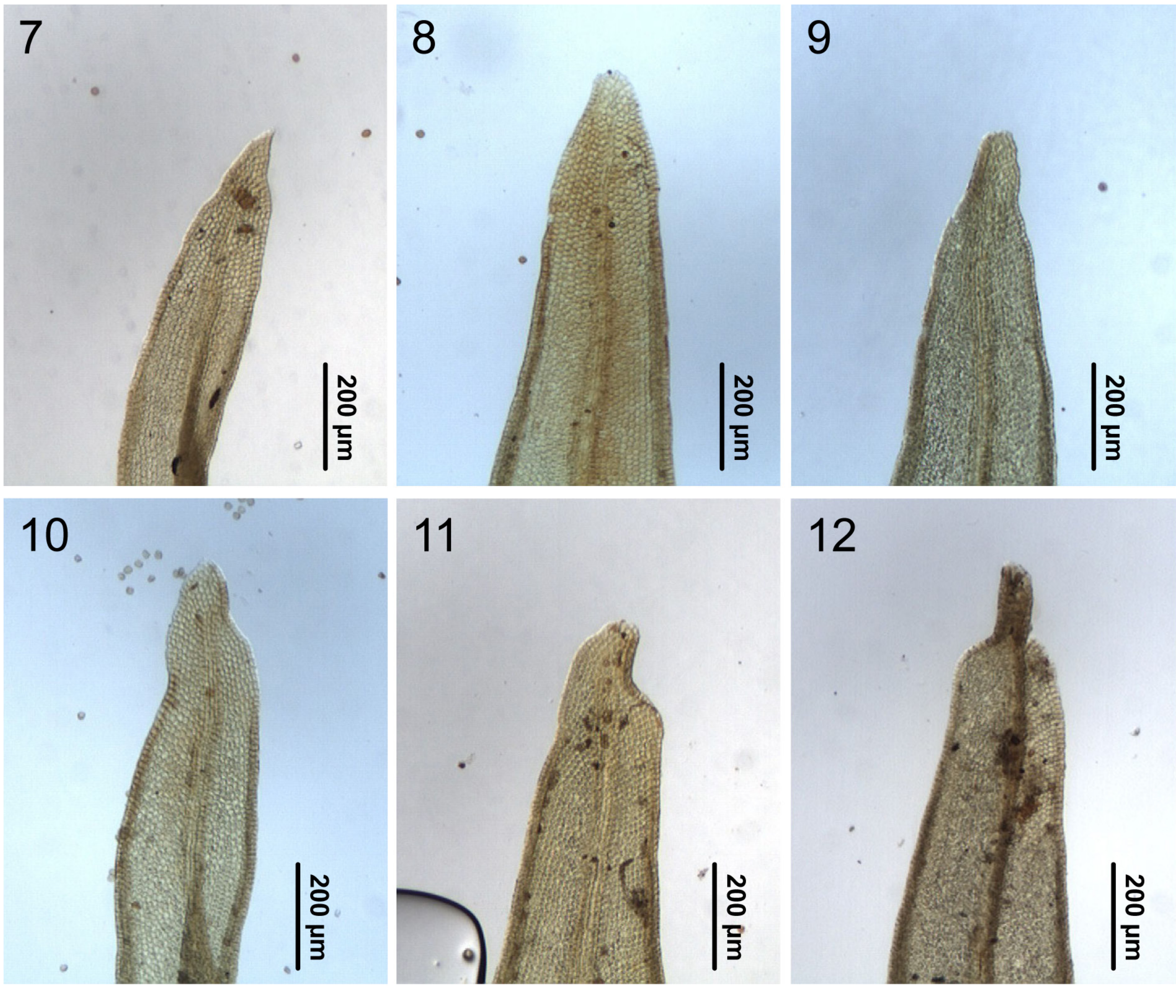
> *Planta usque ad 1.5 cm alta, filidiis in sicco adpressis, lanceolatis, saepe basi ovalibus et ad apicem ligulatis; margine recurvato sed ad apicem plano vel incurvato, canaliculum formanti. Capsula immersa, cylindrica non constrictaque, plerumque vaginula pilifera. Calyptra oblonga vel conica, apice pilosissima. Exothecium octo longitudinalibus angustis vitis quae lato solum duas cellulas habent. Stomata cryptopora in capsulae tertiis (dimidiis) inferioribus disposita. Peristoma duplex, exostoma octo dentium paribus recurvatis, endostoma octo segmentis tenuibus.*

TYPE. U.S.A. CALIFORNIA: San Luis Obispo Co., in chaparral with riverine corridor of oak forest at Queen Bee Campground about 1 mi E of Lapanza Summit, Sec. 3, T.30S., R.16E., 35º21'N, 120º14.5'W, ca. 2400 ft, on moist, diffusely lit bark of live oak, 27 Dec 1979, D. H. Norris 55168 (holotype UC).



Figures IV.1.1–6. *Orthotrichum norrisii*. 1. Dry and empty, narrowly cylindrical capsules showing the narrow, long striae. 2. Operculate, dry capsules with the striae hardly marked; note the plane and rostrate lid, darker than capsule. 3. Exothecium showing narrow, two-celled exothecial bands. 4. View of the lower 2/3 of a capsule; the stomata are located in the lower half of capsule. 5. Peristome. 6. Vaginula showing the long, hyaline and partially biseriate hairs. (1, 3–6 from the holotype, 2 from *D. Toren 975*, SFSU).

Description. *Plants* 0.6–1.0(–1.5) cm tall, in small cushions. *Rhizoids* reddish-brown, smooth, on stem bases and occasionally in old leaf axils. *Stems* well branched, in section rounded-pentagonal with a brownish sclerodermis 2(–3) cells wide. *Axillary hairs* 2–6 cells long, to 150 µm, hyaline above, with 1–2 brownish basal cells. *Leaves* appressed when dry, erect-patent when moist, 1.3–3.0 x 0.3–1.0 mm, somewhat keeled, lanceolate, sometimes with base ovate and lamina lanceolate-ligulate; margins recurved along most of their



Figures IV.1.7–12. *Orthotrichum norrisii*. Variation in leaf apices. (7–11 from the holotype, 12 from *D. Toren 1042*, SFSU).

length; apex rounded, obtuse or acute, with margins plane or with one or both of them incurved, becoming concave or even forming a channel; costa ending below apex, 40– 55 µm wide at base, 30–40 µm wide at midleaf, with two rows of ventral cells; basal leaf cells shortly rectangular to elongate-polygonal, (22–)30–60(–90) x (7–)10–17(–20) µm walls usually thin and straight, sometimes thick and sinuose or even nodulose; marginal basal cells quadrate to shortly rectangular, (8–)11–15(–20) x (10–)12–14(–17) µm; middle and upper laminal cells isodiametric to shortly elliptical, (7–)10–16(–20) x (7–)9–14(–16) µm, with (0–)2–3 simple, slightly prominent papillae. *Gemmae* sometimes present on leaves, brown, cylindrical, 4–7(–15) cells long, 90–160 µm in length.

Goniautoicous. Perigonia lateral; leaves ovate, 0.6–1.0 mm long. Perichaetia terminal; leaves not differentiated. *Vaginula* usually hairy, with partially biseriate hyaline hairs, 250–700(–1800) µm long, sometimes naked; *seta* (0.5–)0.7–1.0 mm long; *capsule* (1.5–)1.7–2.0(–2.2) 3 0.3–0.4 µm, 2–4x as long as the seta, hemiemergent, yellowish, turning brown with age, rightly cylindrical when dry and full of spores, cylindrical and slightly or not constricted when dry and empty, ovoid to widely cylindrical when moist; exothecial cells rectangular, 17–90 x 8– 20 µm, in 8 bands differentiated almost along the whole

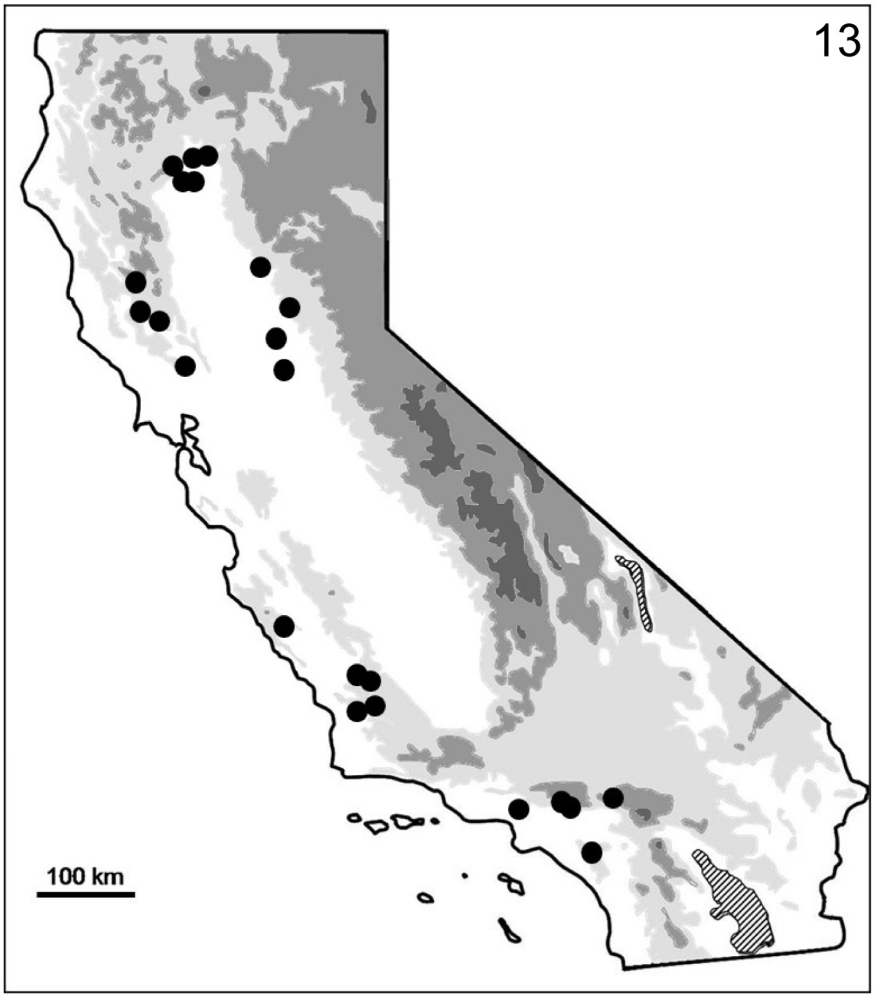


Figure IV.1.13. *Orthotrichum norrisii*. A map of California showing the known distribution of the species.

capsule length, 2(–3) cells wide, cells (16–)20– 60(–80) x (8–)12–28(–35) µm; *stomata* cryptopore, located in the lower 1/3(–1/2) of the capsule, partially to almost completely covered by surrounding cells; *operculum* almost plane, rostrate, orange, with a reddish distinct basal ring, 0.4–0.5 mm in diam., rostrum 150–190 µm long; *peristome* double; *exostome* of 8 pairs of teeth, triangular, pale yellow to orange, recurved when dry, (130–)150–200(–220) µm long; external tooth side (OPL) densely papillose, towards the apex frequently with scattered longitudinal striae; internal tooth side (exostomial PPL) smooth or very slightly ornamented with longitudinal lines at base; *endostome* of 8 hyaline, linear, non appendiculate segments, (120–)140– 200(–220) µm long, smooth on the outside (endostomial PPL), smooth or slightly papillose on the inside (IPL); connective membrane absent or fragmented, ornamentation like that of the IPL. *Spores* slightly papillose, (12–)15–17(–21) µm in diam. *Calyptra* oblong-conic, yellowish, with abundant hairs grouped at the apex.

Etymology. The new species is named in honor of Daniel H. Norris in token of his outstanding contributions to bryology, particularly to the knowledge of Californian mosses. Dr. Norris also provided most of the collections studied for this paper.

Distribution and ecology. Based on herbarium records, *Orthotrichum norrisii* has been collected between 1862 and 1996 throughout California, and it is presently known from 13 counties. Most of the known localities correspond to the lower slopes of the mountainous ranges (Fig. IV.1.13), mainly at 200– 900 m. This epiphytic species grows in Mediterranean-type woodlands and chaparrals, mainly on the bark of evergreen and deciduous *Quercus* species such as *Q. agrifolia* and *Q. douglasii*, but also on other phorophytes in the genera *Aesculus*, *Juglans*, *Salix* and *Pseudotsuga.*

Discussion

Following Lewinsky's (1993) classification, *Orthotrichum norrisii* is a member of subgenus *Pulchella*, because of its capsules with cryptopore stomata, exostome teeth recurved when dry and endostome segments with free apices. This subgenus is a diverse, mainly Holarctic group, with 59 accepted species (Cortini Pedrotti & Lara 2001; Garilleti et al. 2001, 2006a; Goffinet et al. 2007; Lara et al. 1994, 1999a, 1999b, 2000, 2007; Lewinsky 1993; Lewinsky-Haapasaari 1996; Lewinsky-Haapasaari & Norris 1998; Mazimpaka et al. 1999). To date, 19 species in subgenus *Pulchella* are recognized in North America (Vitt 1973, 1994), two of which have been recently described (Garilleti et al. 2001, 2006a). Within subgenus *Pulchella*, *Orthotrichum norrisii* shows a unique combination of qualitative mainly sporophytic characters that affords its recognition at specific rank, and permits its safe identification: capsule narrowly cylindrical when wet and dry (Figs. IV.1.1, 2); eight narrow exothecial bands formed by only two rows (occasionally three at some points) of differentiated cells (Fig. IV.1.3); peristome with eight pairs of exostome teeth and eight endostome segments (Fig. IV.1.5); stomata restricted to the lower part of the capsule (Fig. IV.1.4); plane, rostrate operculum, normally darker than the capsule (Fig. IV.1.2); and vaginula with long, hyaline, partially biseriate hairs (Fig. IV.1.6).

The 33 specimens examined having these characters had been identified as *Orthotrichum tenellum*. This confusion is not surprising since *Orthotrichum norrisii* and *O. tenellum* share the general appearance and significant characters, such as the position of the cryptopore stomata (Fig. IV.1.5), the calyptra with hairs that are mainly apical (Fig. IV.1.1), and the leaf apices plane and obtuse or, frequently, incurved in one or both sides forming folds or a channelled point (Figs. IV.1.7–12) (Cortini Pedrotti & Lara 2001; Piccioli 1932; Venturi 1887). The North and Central American *Orthotrichum bartramii*

R. S. Williams shares these characters (Vitt 1973), but it is a larger moss, with abundant rhizoidal tomentum, and ovate-lanceolate leaves that are abruptly narrowed to the acumen and forming marked shoulders below it (the narrowing is more progressive in both *O. tenellum* and *O. norrisii*, and does not form shoulders below the acumen when it is differentiated). The discrimination of *Orthotrichum norrisii* from both *O. tenellum* and *O. bartramii* is mainly based on the width of the exothecial bands which in the former consist of only two rows (occasionally three) of differentiated cells with thickened and darker cell walls (Fig. IV.1.3), while in the other two mosses the exothecial bands are distinctly wider and composed of 4–6 rows of differentiated cells. This feature is taxonomically relevant within the subgenus Pulchella (Cortini Pedrotti & Lara 2001; Garilleti et al. 2001) where most species have exothecial bands 4–6 cell rows wide.

The exothecial band structure (differentiation degree, length and width) is related to the capsule contraction when dry and thereby it plays a relevant role in spore release. The differentiation degree affects not only the band cell shape and size, but also the organization of cell walls. When observed under polarized microscope (Garilleti et al. 2006a, b; Lara et al. 2008), the birefringence of the exothecial band cells shows that a distinctive, constant organization of cellulose microfibers in these cell walls exists. Accordingly, the intensity of birefringence varies with the degree of differentiation of the exothecial band cells. When the bands are poorly differentiated, and the capsule remains uncontracted when dry, as for *Orthotrichum shawii* (Garilleti et al. 2006b), birefringence is scarcely pronounced, whereas strong bands bright intensely under polarization, like those in *O. anodon* (Garilleti et al. 2006a), *O. patens* and *O. stramineum* (Lara et al. 2008). Similarly, the number of cell rows in the exothecial bands—and consequently the width of the bands—is a qualitative, stable trait that remains invariable through capsule ontogeny. For its constancy and discriminative value the number of differentiated cell rows in the exothecial bands has been largely recognized as a useful taxonomical character for taxa discrimination, being used both in the description of new species (e.g., Brotherus 1898; Garilleti et al. 2001; Goffinet et al. 2007; Lara et al. 2008; Norris et al. 2004; Pluijm 2004; Sotiaux et al. 1998) and in taxonomical revisions (e.g., Cortini Pedrotti & Lara 2001; Lewinsky 1978, 1984a, b; Lewinsky-Haapasaari 1998; Pierrot 1978; Vitt 1973, 1994; Venturi 1887).

In North America only two other species within the group have thin bands of 2–3 cell rows, *O. pulchellum* Brunt. and *O. underwoodii* F. Lara, Garilleti & Mazimpaka. The former is easily distinguished by its exserted capsules with red-orange peristomes, naked calyptrae half-covering the capsules, and leaves more or less contorted when dry. *Orthotrichum underwoodii* is clearly different because of its exostome that has 16 whitish teeth irregularly recurved, its 16 endostome segments, its capsule abruptly contracted to the seta whose apical part is hidden in a hollow, its conic-convex operculum, its short exothecial bands limited to the upper part of capsule, and its leaf margins scarcely recurved, with one side usually plane.

Two other species from Europe have comparable narrow exothecial bands. *Orthotrichum scanicum* Grönvall is very similar to *O. norrisii*, but the European taxon has 16 endostome segments, convex, mammillate opercula (plane and rostrate in *O. norrisii*), and naked vaginula. *Orthotrichum patens* Bruch & Brid. always has acute to acuminate leaf apices, a naked or scarcely hairy calyptra, and its capsule shape is broadly cylindrical and abruptly contracted to the seta.

Additional specimens examined

U.S.A. CALIFORNIA: 1862, *Brewer 1487* (FH); Butte Co., opposite spillway Oroville Dam, 31 Jan 1979, *Norris 52551* (UC); Lake Co., end of Hell's Peak Road, 8 Aug 1973, *Toren 975* (SFSU), near Hell's Peak, Jan 1974, *Toren 1042* (SFSU), ca. 3 mi N of Lakeport, Aug 1974, *Toren 1444* (SFSU), road to Lake Pillsbury near the Slides, Mar 1975, *Toren 1742* (SFSU), along Hwy. 53 about 1 mi S of Hwy. 20, 3 Apr 1976, *Norris 47672, 47688, 47690* (UC); Los Angeles Co., Sawtelle, 1903, *Hasse 3153* (FH), San Jose Hills, Pomona, 1 Mar 1919, *Munz* (FH), San Dimas, Puddingstone, 6 Mar 1928, *Fosberg P13* (FH); Monterey Co., along Sam Jones Road about 1 km E of Piojo Airstrip, Hunter-Leggett Military Reservation, 13 Mar 1996, *Norris 87259* (UC); Napa Co., along shore of Lake Berryessa near intersection of road to Pope Valley, 5 Mar 1977, *Norris 48262, 48268* (UC); Nevada Co., 4.6 mi S of Grass Valley on Hwy. 49 along small creek, 14 Aug 1969, *Vitt 1882* (ALTA); Orange Co., Trabuco Canyon, about 3 mi E of O'Neill Park near Cleveland National Forest boundary, 17 Feb 1975, *Shevock 3988* (UC); Placer Co., along Clover Valley Creek at Sierra College Road, 18 Jan 1994, *Norris 81948* (UC); Sacramento Co., SE of Sacramento between Slough House and Bridge House, 24 Mar 1974, *Weber* (COLO); San Bernardino Co., Waterman Canyon, San Bernardino Mts., 26 Feb 1930, *Fosberg F317* (FH, NY); San Luis Obispo Co., in deep canyon about 1.5 mi W of jct. roads to Black Mtn. and that to Navajo Camp, 28 Dec 1979, *Norris 55260* (UC), along Hwy. 166 about 1 mi W of last crossing of Twitchell Reservoir, 28 Dec 1979, *Norris 55280* (UC), along Hwy. 166 at Clear Creek, Los Padres Natl. Forest, 15 Mar 1993, *Norris 80671* (UC); Santa Clara Co., facing slopes above Uva Creek in Uva Canyon Co. Park S of San Jose, 25 Jun 1977, *Norris 48604* (UC); Shasta Co., drainage canal along the Sacramento River, Redding, 17 Sep 1941, *Richards & Drouet* (FH), along Anderson to Shingletown Rd. at Millville Plains Rd. about 6 mi E of Anderson, 15 Mar 1973, *Norris 23699* (COLO, UC), Little Cow Creek between Bella Vista and Ingot, 19 Jun 1975, *Toren 1919* (SFSU), along Hwy. 299 about 9 mi E of Bella Vista, 19 Mar 1976, *Norris 47590, 47612* (UC), along I-5 about 3 mi N of Tehama Co. line, 3 Mar 1977, *Norris 48216* (UC).

Acknowledgments

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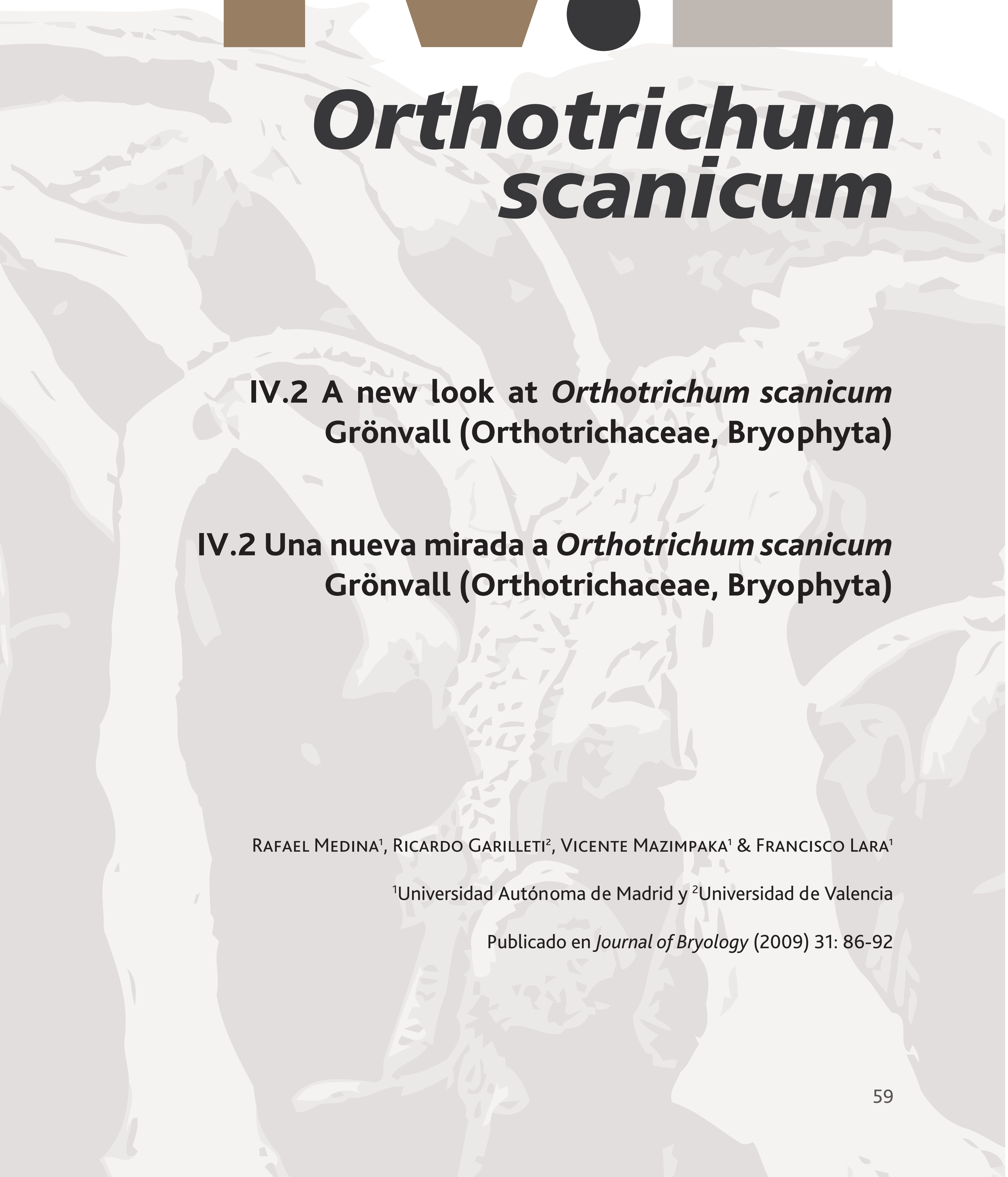

IV.2

Orthotrichum scanicum

IV.2 A new look at *Orthotrichum scanicum* Grönvall (Orthotrichaceae, Bryophyta)

IV.2 Una nueva mirada a *Orthotrichum scanicum* Grönvall (Orthotrichaceae, Bryophyta)

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Abstract

Orthotrichum scanicum Grönvall has been considered a European endemic whose main range corresponded to the central and northern region of the continent, and it is currently included in the 2007 IUCN World Red List as Vulnerable. However, a large number of recent reports and the examination of herbarium specimens have remarkably expanded the limits of its area in the Mediterranean and Caucasian regions. Additionally, the revision of the original collections of *Orthotrichum holmenii* from Kazakhstan shows that this taxon is conspecific with *O. scanicum*. An updated description of the species comprising the known range of its morphological variability is provided, and its exclusion from the World Red List is suggested.

Resumen

Orthotrichum scanicum Grönvall se venía considerando un endemismo europeo con una distribución que abarcaba principalmente el centro y norte del continente y en la actualidad aparece en la Lista Roja Mundial de la IUCN 2007 como Vulnerable. Sin embargo, un gran número de citas recientes y el estudio de especímenes de herbario han ampliado notablemente los límites de su área en las regiones mediterránea y caucásica. Además, la revisión de las colecciones originales de *Orthotrichum holmenii* de Kazajistán indica que este taxón es conespecífico con *O. scanicum*. En este trabajo se proporciona una descripción actualizada de la especie englobando la variabilidad morfológica conocida y se sugiere su exclusión de la Lista Roja Mundial.

Introduction

Since the early descriptions of the taxon, the definition of *Orthotrichum scanicum* Grönvall and its distinction from its closest relatives have been based on a certain number of sporophytic (and a smaller number of gametophytic) characters that are still considered relevant. Thus, in the description of *O. leucomitrium* Bruch & Schimp. *hom. illeg*. based on specimens from central Europe (southern Germany and Austria), Bruch & Schimper (1837) remarked the following character combination: the possession of narrow exothecial bands, a very convex operculum, large exostome teeth, an endostome of 16 long, equal, segments and a calyptra with some hairs. Later, in the legitimate publication of the taxon, Grönvall (1885), based on Scandinavian material, distinguished *O. scanicum* by means of a group of characters, among which he emphasized the endostome of 16 sub-equal, slightly papillose endostome segments.

The characters used to define *Orthotrichum scanicum* are qualitative and allow a clear differentiation of the taxon. In fact, almost no superfluous names have been published for this moss during a period of great bryological activity, the whole 19th and most of the 20th century. Only Venturi (1873) described the variety *Orthotrichum scanicum* var. *elatum*, and Grönvall (1887) proposed the subordination of his own *O. scanicum* to the illegitimate name *O. leucomitrium* as a variety (*O. leucomitrium* var. *scanicum*), thus making an invalid combination.

Until the 1990s, *Orthotrichum scanicum* has been considered a rare European species, whose range was confined to central and northern countries of the continent (Düll, 1985). Its relatively reduced range, and especially the extinction of populations throughout its known area, led to its inclusion in the Red Data Book of European bryophytes (ECCB, 1995) and in the 2000 and 2007 IUCN World Red Lists of bryophytes (Tan et al., 2000; Hallingbäck et al., 2007). Nowadays, its global conservation status is set as Vulnerable (VU).

More than a century after the description of *Orthotrichum scanicum*, two related taxa were published. Both came from localities that are geographically and climatically far from each other, and from the classical area of *O. scanicum*: *Orthotrichum holmenii* Lewinsky-Haapasaari (Lewinsky-Haapasaari, 1996) from Kazakhstan and *O. lewinskyae* F. Lara, Garilleti & Mazimpaka (Garilleti, Lara & Mazimpaka, 1997) from northern Morocco. The validity of the latter was questioned by Sotiaux, Sotiaux & Pierrot (1998), who suggested that the differences pointed out in the protologue of *O. lewinskyae* – mainly quantitative – could fall within the variation of *O. scanicum*. The discovery of new populations in the Iberian Peninsula and SW France (in areas that are climatically intermediate between central to northern Europe and northern Africa) allowed

Figure IV.2.1. *Orthotrichum scanicum*: (1) detail of the habit; mature capsules with opercula and one calyptra where an apical hair can be observed; (2) sporophyte after spore release; (3) view of the exothecium in bright field; narrow 2-cells wide exothecial bands are shown; (4) the same detail, under polarized light; (5–10) variability of leaf apices; (7,8) variability of leaf papillosity. 1, 2, France: Fabian (VAL-Briof); 3, 4, France: Néouvielle (VAL-Briof). 5–10, Morocco: Jbel Tazzeka (Authors' Herbarium at UAM). 11, Italy: Boscolungo (Authors' Herbarium at UAM). 12, Spain: Mirantes de Luna (Authors' Herbarium at UAM).

substantiation of the suggestion by Sotiaux et al., (1998), and subsequently in Draper et al., (2003) the two names were formally synonymized.

Regarding *Orthotrichum holmenii*, the characters used for its discrimination were mainly qualitative (Lewinsky-Haapasaari, 1996), so it seemed to be a well-defined taxon, easy to distinguish from *O. scanicum*: (1) endostome segments strongly papillose; (2) capsules with seta 0.8–1.0 mm long, sometimes bent to the side; (3) calyptrae completely naked; (4) leaf apices acuminate to acute, with margins entire or slightly crenulate from protruding papillae; (5) bifurcate papillae on leaves, and (6) presence of long gemmae on leaves, some of them branched. Two additional quantitative characters were provided to distinguish this taxon from *O. scanicum*: (7) the almost free to half-covered cryptopore stomata, compared with the almost completely covered ones of *O. scanicum*, and (8) the smaller upper leaf cells (8–13 mm width in *O. holmenii*, 12–14 mm in *O. scanicum*). At the time of its publication, these traits appeared solid and exclusive, supporting the distinction of specimens from Europe and Kazakhstan.

However, finds of new populations of *Orthotrichum scanicum* throughout a wide circum-tethyan area, including southern and eastern Europe (Garilleti et al., 1999; Lara et al., 2001, 2003), northern Africa (Garilleti et al., 1997; Draper et al., 2003), Turkey (Erdağ, Kürschner & Parolly, 2004), and Georgia (a sample collected by Brotherus and kept at TR-Venturi!) suggested a review of the variability of the species. Conclusions from this broad study have shown that the morphological variability of *O. scanicum* is larger than previously considered, and is continuous throughout the species' area. The characters used to define *O. holmenii* fall within this variability, and merely represent variation extremes. (1) Endostome segments of *O. scanicum* are usually only slightly papillose, but some specimens with high and dense papillae have been found in Turkey and Spain; the samples from Kazakhstan have the most strongly papillose endostomes but this character state can be considered the extreme of the variability; (2) the seta length of specimens described as *O. holmenii* (0.8–1.0 mm) falls within the range of other studied specimens of *O. scanicum* (0.3–1.3 mm); bent capsules appear only in a small proportion of the plants from Kazakhstan and have occasionally been observed in specimens of *O. scanicum* from other areas; this phenomenon seems to be related to the seta length, being most frequent in relatively longly exserted capsules; (3) calyptrae are more frequently naked in the specimens from Kazakhstan, but sparse, inconspicuous hairs are found in some of them, and specimens of *O. scanicum* with naked calyptrae are occasional in Europe and Morocco. (4) The forms of leaf apices described for *O. holmenii* (acuminate to acute, entire or crenulate) fall within the variability of *O. scanicum* throughout its area of ocurrence; indeed, the typical *O. scanicum* apices with folds and channelled points are also present in the three Asian collections. (5) High and bifurcate papillae on the leaf cells are a remarkable feature of the specimens from Kazakhstan, but they were also found in *O. scanicum* from north-western Spain and north-eastern Turkey in harsh environments (Fig. IV.2.12); this suggests that such papillae could be a response to environmental conditions and thus have no taxonomic value. (6) Fusiform or cylindrical gemmae have occasionally been observed on leaves of *O. scanicum*, but branched gemmae as those described in the protologue of *O. holmenii* have never been found in this species; according to Lewinsky-Haapasaari (1996), such branched gemmae were only found in one of the three original collections (*Magill 10308*); re-examination of this sample showed that the gemmae were actually produced by individuals of *Nyholmiella obtusifolia* (Brid.) Holmen & Warncke present in the specimen. (7) The stomata of the samples from Kazakhstan are indeed scarcely covered by the exothecial cells in most cases, but again this situation should be understood as the extreme of a continuous gradient for this character; some *O. scanicum*

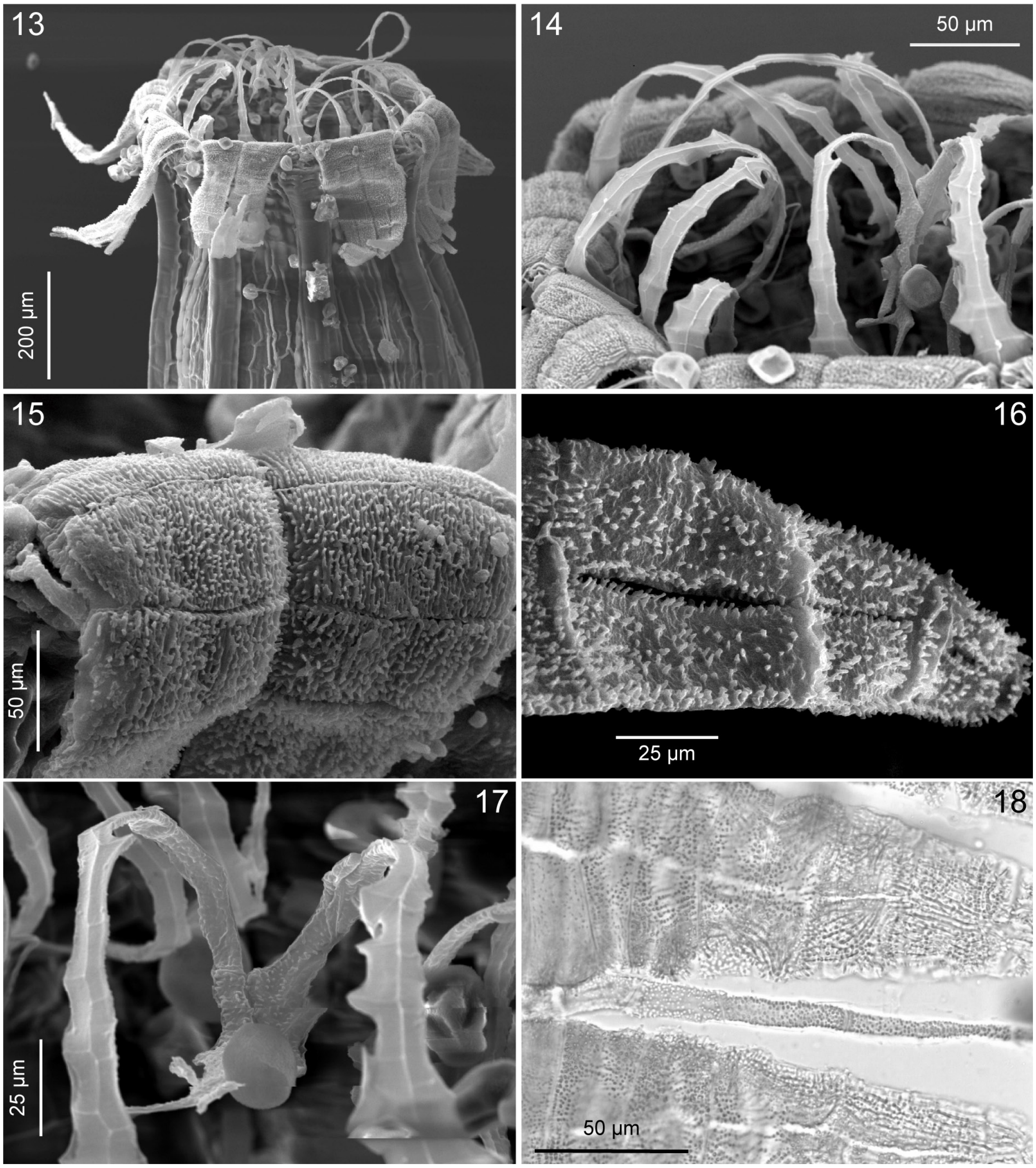

FIGURE IV.2.2. *Orthotrichum scanicum*: (13) SEM view of capsule mouth; (14) SEM view of endostome; (15) SEM detail of the ornamentation of the inner basal side of the exostome; (16) SEM detail of the ornamentation of the inner distal side of the exostome; (17) SEM view of the ornamen¬tation of the endostome (both faces); (18) Appearance of the peristome ornamentation in bright field. (13, 14, 17) Morocco: Ketama, (Authors' Herbarium at UAM). (15, 16), Morocco: Azrou (Authors' Herbarium at UAM). (18), France: Fabian (VAL-Briof).

specimens from Morocco frequently show broadly opened stomata; conversely, half covered stomata can be seen in *O. holmenii*; moreover, as has been observed in most of the cryptopore *Orthotrichum* species, it is common to find that in a single capsule the lower stomata are more covered than the upper ones; thus, we conclude that the degree to which adjacent exothecial cells cover cryptopore stomata can be used as a morphological trend, but it is not consistent as a qualitative, differentiating character.

To sum up, the Asian samples show many features which do not completely match the most typical European *Orthotrichum scanicum* plants. However, these traits can be regarded as the extremes of the whole variation range observed in the studied collections, and they are not exclusive of the specimens from Kazakhstan, so, they cannot be used for specific differentiation. It has not been possible to find new characters that support the validity of *O. holmenii*, therefore we suggest it should be considered conspecific with *O. scanicum*.

The extension of the recognized morphological variability of *Orthotrichum scanicum* after the examination of a large number of known populations (103 herbarium specimens) and the synonymization of *O. holmenii* make an updated description of the taxon necessary.

Description

Orthotrichum scanicum Grönvall, Bidrag till Kännedomen om de Nordiska Arterna af de Båda Löfmoss-slägtena *Orthotrichum och* Ulota 13. 1885. Type: Sweden. Sk. Börringe (Holotype: S!).

Orthotrichum leucomitrium Bruch & Schimp. in B., S. & G., Bryologia Europaea 3: 68. 220 (fasc. 2–3 Mon. 26). 1837. *hom. illeg*. Type: Carlsruhe ... Baden ... Dahn (in Vogeso infer.) ... Bipontem ... Monachium et Salisburgam.

Orthotrichum leucomitrium Bruch & Schimp. var. *elatum* Vent., Hedwigia 12: 24. 1873. Type: In sylvis vallis Rabbi, (Holotype TR!, cf. Garilleti, Lara & Mazimpata, 2007).

Orthotrichum holmenii Lewinsky-Haapasaari. The Bryologist 99(1): 1–5. 1996. Type: Kazakhstan. Alma Ata. Alma Ata Oblast, Alma Ata Wilderness Preserve, Levii Talgar Valley, entrance to preserve, 1200 m, *Allen 10729a* (Holotype MO!) *syn. nov*.

Orthotrichum lewinskyae F. Lara, Garilleti & Mazimpaka in Garilleti, F. Lara & Mazimpaka. Journal of Bryology 19: 457–464. f. 1–16. 1997. Type: Morocco. Azrou, 'Cèdre Gouraud' cedar wood, 1700 m a.s.l., *Albertos, Garilleti, F. Lara & Vergara s.n.* (Holotype MA!).

Plants (0.3 –)0.5–1.5(–2.0) cm tall in small loose cushions or scattered among other *Orthotrichum* species, yellowish green to dark olive green. *Rhizoids* reddish-brown, smooth or very sparsely papillose, on stem bases, occasionally in axils of old leaves. *Stems* well branched at bases, pentagonal to sub-rounded in section with a 2–3 layered brown sclerodermis. *Axillary hairs* 4–7 cells long, up to 165 µm, hyaline except 1–2 brownish basal cells. *Leaves* erect to appressed, somewhat flexuose when dry, erect-patent when moist, (1.0–)1.5–3.0(–3.5) x (0.3–)0.5–1.0 mm, somewhat keeled, linear-lanceolate to oval-lanceolate, often with a wider oval base, margins entire, recurved from base to near apex; leaf apices very variable, acute to somewhat obtuse, entire or somewhat denticulate, very often apiculate, with one or both margins incurved or folded, forming a channel, rarely plane; costa 35–50(–60) µm wide at middle lamina, with two rows of ventral cells, ending below leaf apex; basal leaf lamina cells rectangular (polygonal) and variably elongate, smooth or rarely papillose, (8–)15– 70(–80) x (5–)8–15(–20) µm, with cell walls 2– 4 µm thick, sometimes slightly nodulose; marginal basal cells elongate, 10 –40(–60) x 5–18 µm; middle and upper leaf lamina cells rounded-isodiametric to slightly rectangular-elongate, and (5–)10–20(–30) x 5–15(–20) µm, with (0–)1–3(–4) papillae, papillae usually not prominent but sometimes reaching 10 µm and bifurcate. *Gemmae* fusiform to cylindrical rarely present on leaves, brown or greenish, of 4–10 cells, 50–120 µm long.

Goniautoicous, rarely cladoautoicous. *Perigonia* lateral, perigonial leaves ovate with acute apex, 450–700 µm long. *Perichaetia* terminal; perichaetial leaves not distinct; *vaginula* 0.7–0.8 mm, naked or very rarely with scattered hyaline hairs. *Calyptra* cylindric to campanulate, pale yellowish, longitudinally plicate, with sparse short hairs often grouped towards apex, more rarely completely naked. *Seta* (0.3–)0.5–0.8(–1.3) mm long. *Capsule* 1.5–2.0(–2.5) x (0.2–)0.3–0.5(–0.6) mm, (2–)3–4 times longer than seta, immersed to semiemergent, usually yellowish, more rarely reddish or dark green, turning brown with age, ovoid when moist, cylindrical when dry and full of spores, cylindric but somewhat constricted below mouth when dry and empty; exothecial cells rectangular-elongate, hyaline or yellowish, 15–60(–90) x 7–30 µm; eight narrow exothecial bands differentiated along most of capsule length, 50–70(–85) µm wide, formed by 2(–3) rows of cells, some of the cells being occasionally duplicate; exothecial band cells 12–70(–90) x 14–35 µm, darker and with thicker cell walls; *stomata* cryptopore in lower 2/3 of the capsule, partially to almost entirely covered by surrounding cells, very rarely almost free. *Lid* convex, orange, mamillate to rostrate, rarely plane-conic, brownish or with a darker basal ring, 0.45–0.65 mm in diameter. *Peristome* double. *Exostome* of 8 pairs of teeth, often irregularly splitting when old, triangular, pale yellow to orange, recurved when dry, (200–)230–270(–310) µm long; external tooth side (OPL) ornamented with high, dense or rarely scattered papillae, sometimes forming short vermicular lines towards apex; internal tooth side (exostomial PPL) with abundant longitudinal lines at base and scattered papillae towards apex. *Endostome* of 16 segments; principal segments

1–2-seriate, linear, variably appendiculate, 180–250(–300) µm long; intermediary segments 1(–2) seriate, similar to the principal segments in shape and length or rarely shorter, at least 50 µm long; external segment side (endostomial PPL) smooth or slightly papillose, internal segment side (IPL) slightly papillose, occasionally with high papillae more or less densely disposed; connective membrane present, ornamented as segments. *Spores* papillose, (12–)15–23(–31) µm in diameter.

Discussion

Morphological variability and differentiation. *Orthotrichum scanicum* is defined by a number of mainly sporophytic characters that are constant or almost so throughout its distribution range. The most remarkable are: (1) the thin exothecial bands, 2(–3) cells wide, whereas the most frequent state in the genus is bands 4–6 cells wide (a discussion of the constancy and validity of this character is given in Medina et al., 2008); (2) the peristomial configuration consisting of an exostome of 8 pairs of teeth with a tendency to split and an endostome of 16 subequal segments, somewhat shorter than the teeth, variably papillose; (3) the convex lid; and (4) the position of the cryptopore, partially covered stomata, restricted to the lower 2/3 of capsule. The most relevant gametophytic character is the shape of the leaf apices (Fig. IV.2.5-10), which are irregular in outline and often have partially incurved margins that form distinctive folds and channeled uppermost leaf portions.

The Eurasiatic *Orthotrichum* species most likely to be confused with *O. scanicum* are *O. tenellum* Bruch ex Brid. and *O. hispanicum* F. Lara, Garilleti & Mazimpaka. Both have differentiated exothecial bands that are 4(–6) cells wide. In addition, *O. tenellum* has a different peristome configuration (8 tooth pairs, without tendency to split, and 8 endostome segments) and stomata only in the lower third of the capsule, while *O. hispanicum* has leaf apices without any differentiation.

Within the subgenus *Pulchella* some other *Orthotrichum* species exhibit narrow exothecial bands of 2(–3) cells width: *Orthotrichum pulchellum* Brunt. can be differentiated by its fully exserted capsules, reddish-orange peristome and its stomata mainly placed in the upper half of the capsule *Orthotrichum patens* Bruch ex Brid. has straight leaves when dry, with acute, non-differentiated apices, an 8-segment endostome, exostome teeth remaining paired even after getting recurved, capsules brown-orange when young, rarely pale brown (never yellowish) and a vaginula that has generally some long hairs (Lara et al., 2008). The recently described *Orthotrichum norrisii* F. Lara, Medina

& Garilleti (Medina et al., 2008) is easily distinguished from *O. scanicum* by a peristome of 8 exostome teeth that do not split and an 8-segment endostome, a plane lid and a truly cylindric capsule when moist.

Distribution and conservation status. The known distribution of *Orthotrichum scanicum* spans a great part of central Europe, southern Fennoscandia, the Mediterranean basin (including northern Africa), the Balkans, Georgia and Kazakhstan. It seems to have its optimum in mountainous areas of the Mediterranean Region, where it is frequent and abundant and colonizes several phorophytes (Draper et al., 2003; Erdağ et al., 2004; Garilleti et al., 1997; 1999; Lara et al., 2001; 2003).

According to the Red List of the Bryophytes of the World (Hallingbäck et al., 2007), *Orthotrichum scanicum* is considered Vulnerable (VU) under the 1994 IUCN criteria: "The population is suspected to have declined by at least 20% over the last 15 years (three generations) because host trees have been cut and air pollution has lowered the vitality of this species". Despite the recent publication of the new version of the IUCN Red List, data concerning the status of *O. scanicum* are those used and published in the year 2000 version of the Red List (Tan et al., 2000, mainly based on ECCB, 1995). Since the initial evaluation of the conservation status of this species our knowledge of the distribution of *O. scanicum* has remarkably improved. This moss has been collected in many new localities and re-found in other ones. Moreover, the size of several known populations is significant, so it seems previous considerations were based on imperfect knowledge regarding its status. *Orthotrichum scanicum* is not a rare moss in the mountains of the Mediterranean and the Caucasian regions, and it can be locally abundant in some forests, such as those of the Rif mountains in Morocco (Draper et al., 2003), or frequent in large areas, such as the Pyrenees (Garilleti et al., 1999), northern Greece (Lara et al., 2003) or northern Turkey (unpublished data). Although locally its populations may be declining due to destruction and pollution of forests, the updated knowledge of this taxon suggests that it should no longer be considered globally vulnerable. However, it could still be locally threatened (Plášek & Mudrová 2006; Werner, Hans & Mahévas 2007).

Selected studied specimens

First country reports of *Orthotrichum scanicum* are marked with an asterisk (*). When no herbarium acronyms are included, specimens are kept in the authors' herbarium at Universidad Autónoma de Madrid.

FRANCE. Hautes-Pyrénées: Néouvielle, Couplan fir-forest, *Draper* & *Albertos* 21 June 2002 (VAL-Briof); Fabian, Camping Fouga, *Draper* & *Albertos*, 23 June 2002 (VAL-Briof). ***GEORGIA.** Achalzich, *Brotherus*, 7 June 1881 (TR-Venturi). **GREECE. Ipiros:** Ioanina, Vikos Gorge, between Aristi and Papingon, *Lara* & *Mazimpaka* 30 July 1999; **Sterea Ellada:** Fokida, Brallos, towards Iti, *Lara* & *Mazimpaka* 27 July 1999. **ITALY. Pistoia:** Boscolungo, Cortini, *Lara* & *Pedroti* 3 December 1999; **Sicilia:** Piano Zucchi, *Garilleti, Lo Giudice* & *Mazimpaka* 9 September 2000. ***KAZAKHSTAN. Alma Ata:** Alma Ata Oblast, Alma Ata Wilderness Preserve, Levii Talgar Valley, entrance to preserve, *Allen 10729A* (MO, holotype of *O. holmenii*); Almatiska Region, Talgar City District, 30 km W of Alma Ata, *Magill 10308 p.p.* (MO); Alma Ata State Reserve, Zolatofts Spring, *Magill 10557 p.p.* (MO). **MOROCCO.** Azrou, «Cèdre Gouraud» cedar wood, *Albertos et al*., 2 April 1994. (MA-Musci, holotype of *O. lewinskyae*); Ketama, base of Jbel Souk, Tahomar, *Lara et al*. 15 March 1997; Ascension to Jbel Tassaot, *Draper & Medina* 10 June 2004; Ascension to Jbel Tazzeka, *Mazimpaka et al*. 21 June 1997; **PORTUGAL. Guarda:** Serra da Estrela, Covão da Ametade, *Garcia* 9 October 1999, (LISU). **ROMANIA.** Eastern Carpathians, Bicaz Gorge, F. *Lara* 17 August 2003; **SPAIN. León:** Mirantes de Luna, *Lara et al.* 23 July 1996; **Guadalajara:** Chequilla, Piedra del Cuervo, *Draper, Medina & Pokorny* 4 June 2004; **SWEDEN: Dalsland:** Dalskogs sn. på trad vid Dalskog jernvagsstation, *P. A. Larson* 24 June 1923 (S); **Skåne:** Börringe, *Grönvall* 1 July 1884 (S, holotype of O. scanicum); **TURKEY. Trabzon:** Track next to Uzungöl, *Lara, Medina & Mazimpaka* 9 July 2005; *Bolu:* Yedigöller Milli Parkı, *Lara, Medina & Mazimpaka* 19 July 2005.

Acknowledgments

We are grateful to the curators of the herbaria MO, S and TR, and to Tomas Hallingbäck for translating the Swedish diagnosis of O. scanicum and for providing some of Grönvall's bibliography. Dan H. Norris and Lars Hedenäs are thanked for stylistic suggestions. This work was funded by the Spanish Ministerio de Educación y Ciencia, grants CGL2004-03513 and CGL2007-61389.

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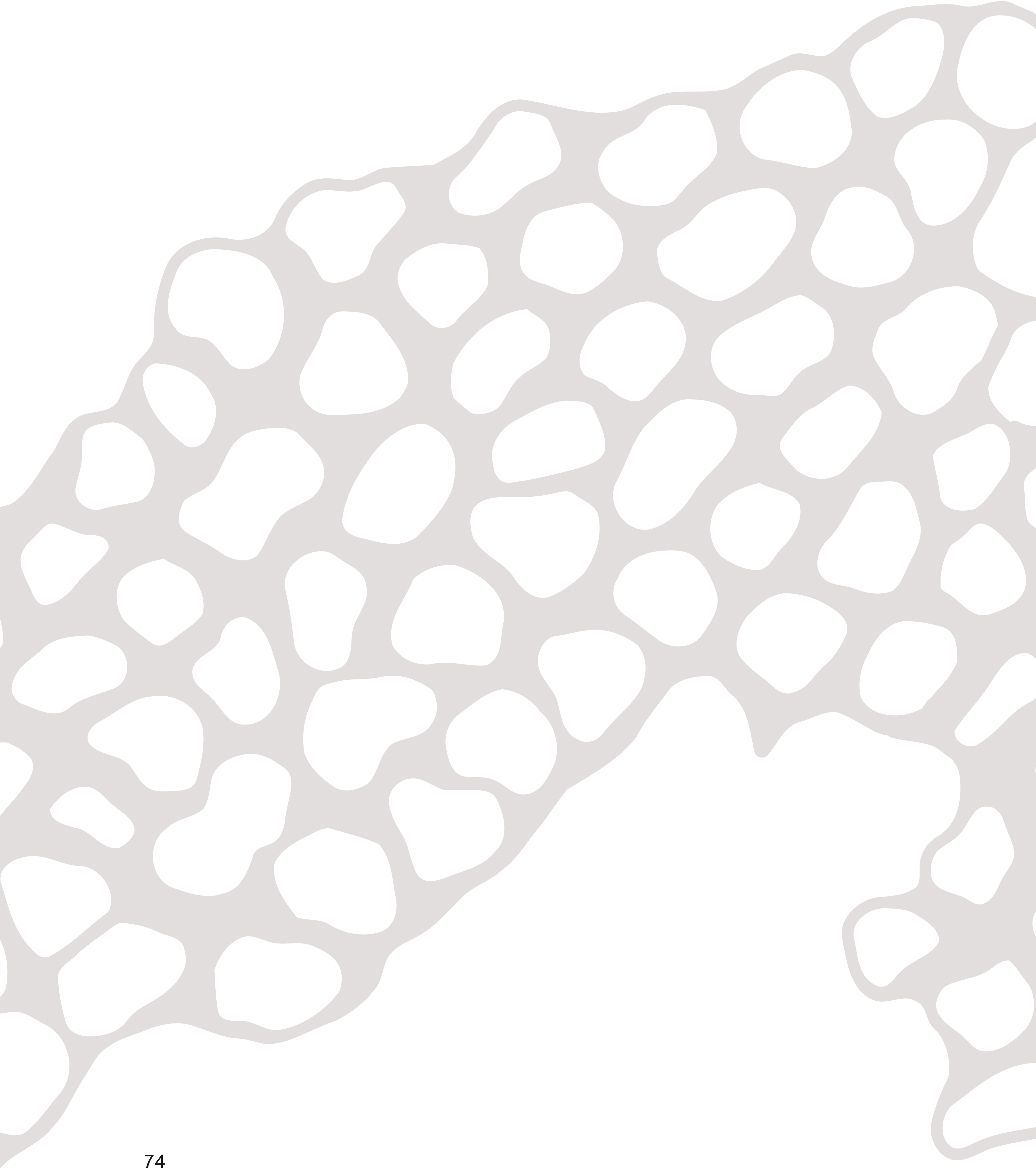

IV.3

Orthotrichum pilosissimum

IV.3 *Orthotrichum pilosissimum* (Orthotrichaceae), a new moss from arid areas of Nevada with unique axillary hairs

IV.3 *Orthotrichum pilossisimum* (Orthotrichaceae), un nuevo musgo de zonas áridas de Nevada con un tipo único de pelos axilares

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Abstract

Orthotrichum pilosissimum is described from herbarium material and recently collected specimens in the state of Nevada. Its specific epithet refers to the diagnostic hyper-developed axillary hairs, which are frequently longer than 1 mm, multiseriate and branched, covering most of the stems surface and sometimes protruding from the foliose shoots. These hairs are not only unique in the genus but also among mosses, providing a reliable diagnostic character for the new taxon. Additional gametophytic and sporophytic characters that contribute to its taxonomic delimitation are also given.

Resumen

Se describe *Orthotrichum pilosissimum* a partir de material de herbario y especímenes de reciente recolección en el estado de Nevada. Su epíteto específico hace referencia a los característicos pelos axilares muy desarrollados, que frecuentemente son mayores de 1 mm de longitud, ramificados y multiseriados, cubriendo en su mayor parte la superficie de los caulidios y a veces sobresaliendo de los vástagos foliosos. Estos pelos no sólo son únicos en el género, sino entre todos los musgos por lo que suponen un carácter diagnóstico fiable para el nuevo taxón. También se aportan caracteres adicionales, gametofíticos y esporofíticos, que contribuyen a su delimitación taxonómica.

Introduction

During the revision of *Orthotrichum specimens* at UC, a voucher from Santa Rosa Range (Humboldt Co., Nevada) attracted our attention due to its exceptionally long and branched axillary hairs, forming a high and dense tomentum covering the stems. This feature had never been reported within the Orthotrichaceae. The specimen was collected in 1973 (*D. H. Norris 24481*, UC) on *Populus* bases and was labeled as *O. tenellum* Bruch ex Brid. It showed some other distinct traits such as the acuminate to subulate leaf apices, but due to its reduced size and poor preservation state, a taxonomic decision was postponed, in particular until the hyper-development of the axillary hairs could be shown to be a constant characteristic of this moss, and not merely a local teratological manifestation.

A stay in Berkeley in Fall 2008 gave us the opportunity to resume the investigation on this odd moss. A prospecting trip was carried out in Central and Northern Nevada, where this moss was found in two localities: one corresponding to the original collection, where this *Orthotrichum* was growing on *Populus* bases and roots as well as on slate, and an additional locality southwards, in Mineral Co., where the species was collected on granitic rocks.

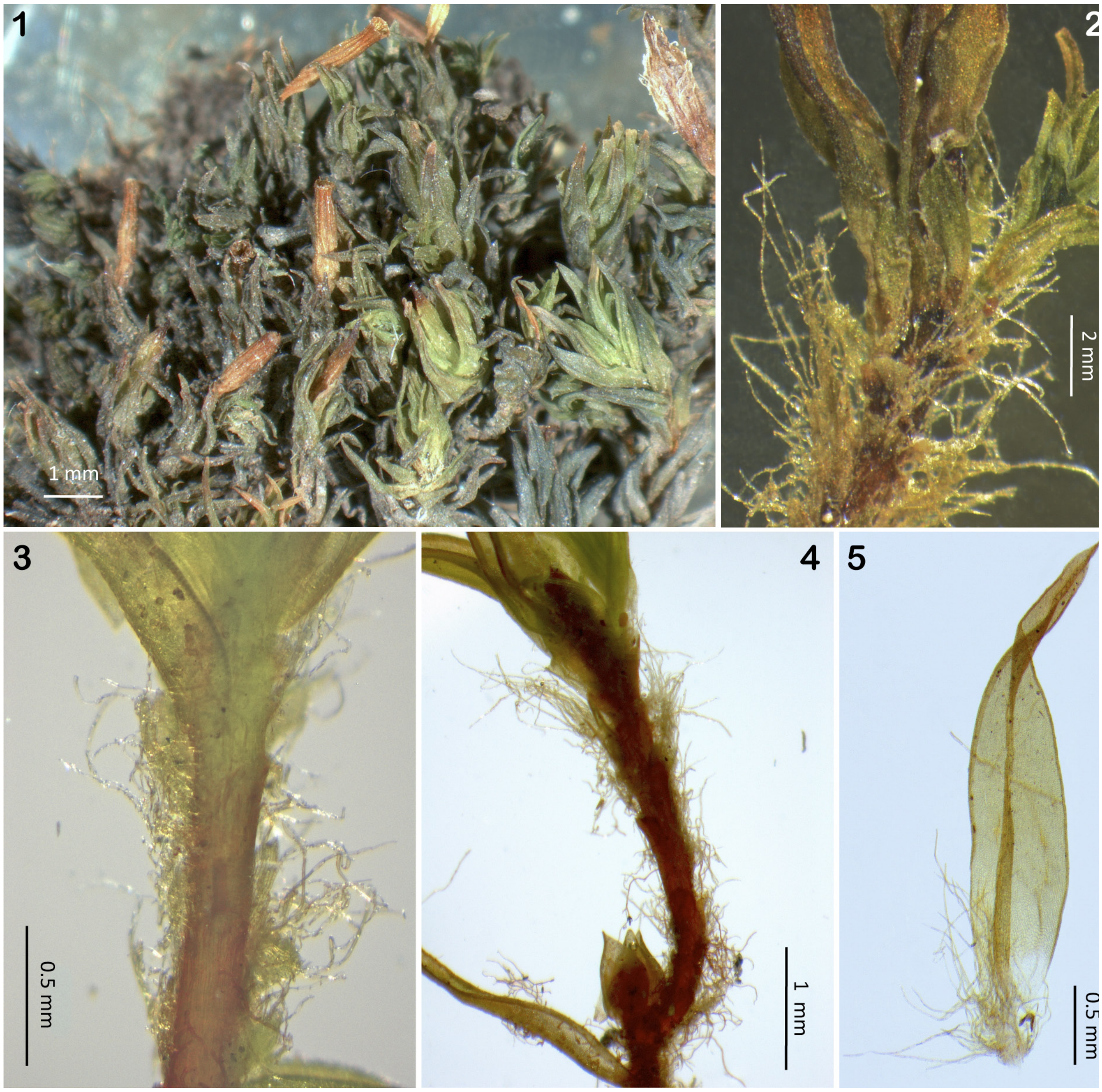
Additionally, the study of herbarium materials kept at CAS and UC yielded four new localities from the state of Nevada. All these specimens share a distinct and unique combination of characters with the extraordinary development of axillary hairs being the most notable trait.

Description

Orthotrichum pilosissimum R. Medina, F. Lara & Garilleti, sp. nov. Figs. IV.3.1–27

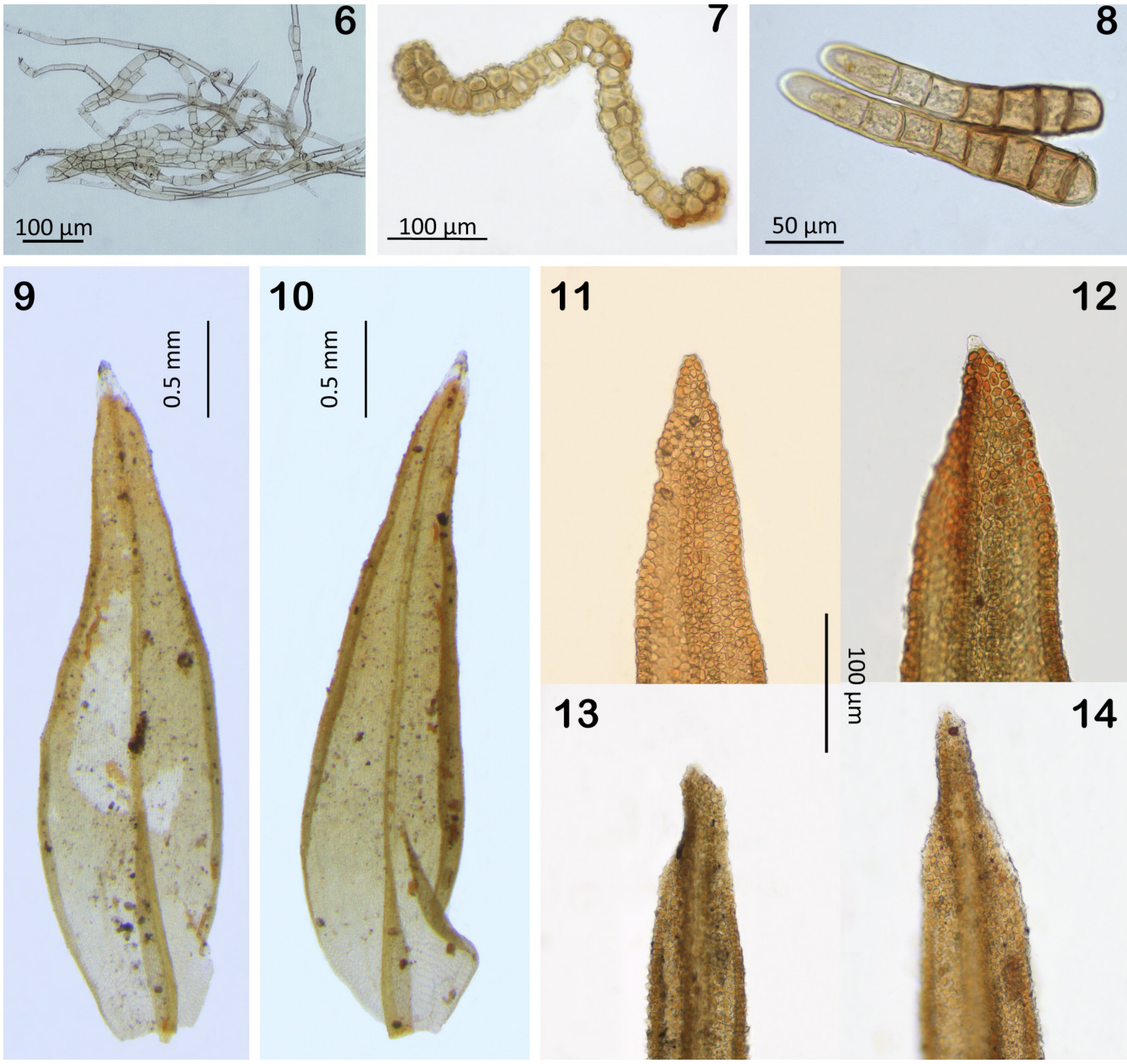
> *Species insignis axillaribus trichomatibus suis longissimis (ad 1.8 mm longis) basi ramificantibus, super caulibus tomentum formantibus. Filidia in sicco adpressa, linearlanceolata, apicibus acuminatis vel subulatis; lamina monostrata partimve bistrata, saepe propagulis munita; marginibus recurvatis vel revolutis, sed ad apicem planis vel incurvatis, longum fragilemque canaliculum formantibus. Vaginula nuda. Capsula immersa vel emergens, valde constricta infra orem post sporosim. Stomata cryptopora in capsulae dimidio inferiore locata. Peristoma duplex, exostoma octo dentium paribus recurvatis, endostoma octo segmentis tenuibus.*

TYPE: **USA. NEVADA**: Humboldt Co., Humboldt National Forest, Santa Rosa Mts., Buffalo Creek, 41º28'34" N, 117º44'49" W, 1530 m, on base and roots of old *Populus balsamifera* L. and on slates, 29 Oct. 2008, *R. Garilleti, F. Lara, J. R. Shevock & B. Albertos* (holotype: CAS; isotypes: NY, VAL, and herbarium of Universidad Autónoma de Madrid).



FIGURES IV.3.1–5. *Orthotrichum pilosissimum*. 1. Habit. 2–3. Stems with removed leaves showing hyper-developed axillary hairs. 4. View of a defoliated hairy stem and a terminal perigonium with a lateral branch arising immediately below it. 5. Leaf with attached axillary hairs. (1 from holotype; 2–4 from Humboldt Co., Anchorite Hills, *Lara et al*. Oct. 2008; 5 from *Norris 24481*).

Description. *Plants* growing in dense, compact tufts, glaucous-green to blackish, 0.4–0.7(–1.2) cm long; *rhizoids* at base and lower part of stems, often growing densely, brown-reddish, smooth to slightly rough. *Stems* subpentagonal in section, with sclerodermis (1–)2–3 cells wide; stem surface and leaf axils densely covered by distinct *axillary hairs* (0.4–) 0.7–1.2(–1.8) mm long, multiseriate and branched at base, uniseriate above, formed by hyaline thin-walled cells, with a few short, brown basal cells, very often forming dense fascicles; ordinary axillary hairs very scarce but also present, 3–6 hyaline cells long, with 1–2 short, brown basal cells, 75–170 µm long. *Leaves* linear-lanceolate, wide at base,



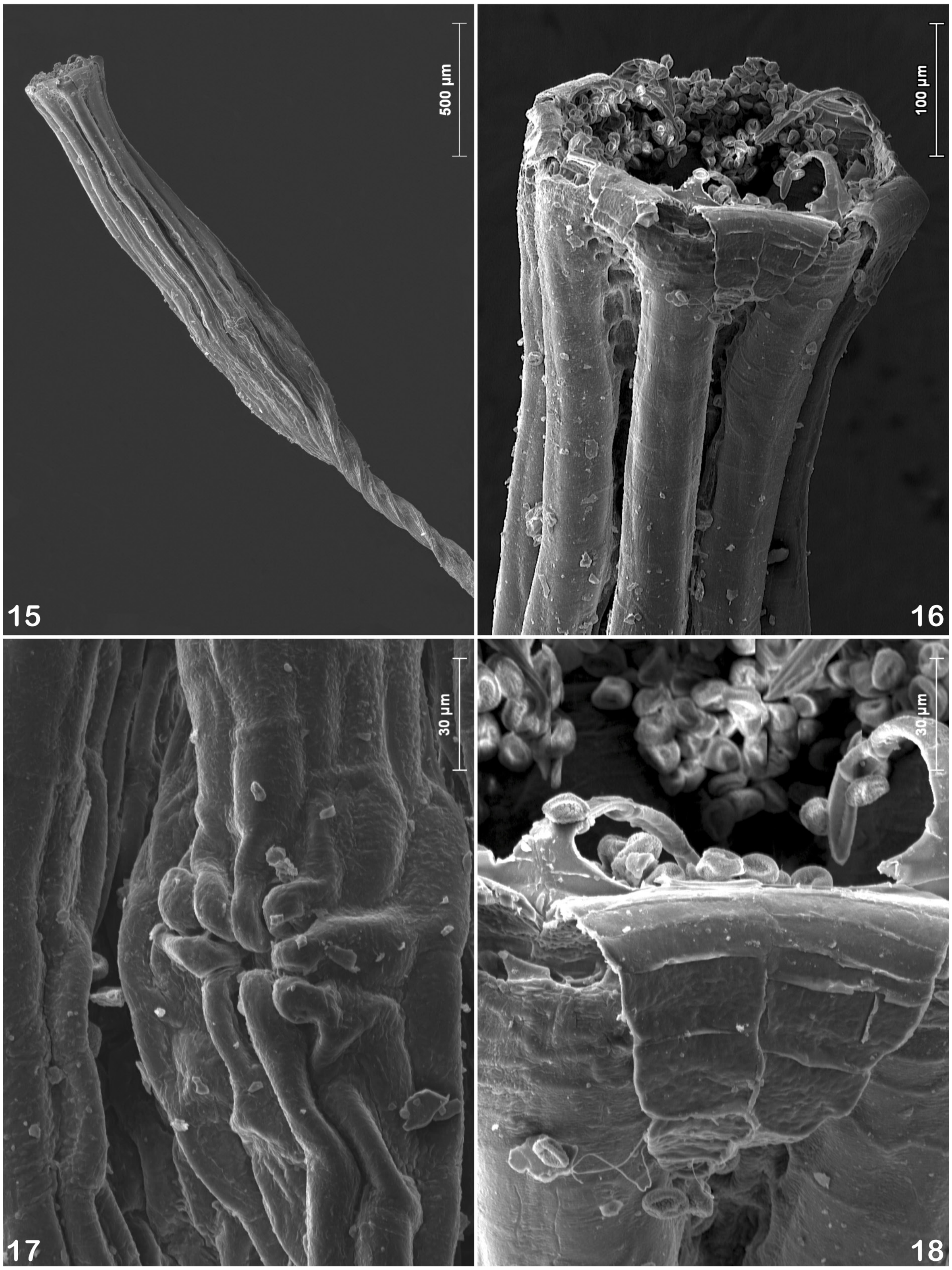
Figures IV.3.6–14. *Orthotrichum pilosissimum*. 6. Detail of a multiseriate axillary hair. 7. Cross section of a leaf showing biseriate strands and margins. 8. Brood-bodies. 9–10. Leaves. 11–14. Leaf apices. (6, 13–14 from holotype; 7–8, 11–12 from *Shevock 21581;* 9–10 from *Shevock 22397*).

appressed and slightly contorted when dry, erect-patent when moist, 2.0–3.0(–3.75) x 0.4–0.8(–1.0) mm, keeled in section, unistratose or rarely with scattered bistratose ridges towards the upper part; margins recurved from base to almost leaf apex, in the upper half often revolute and sometimes bistratose; leaf apex acuminate to subulate, occasionally with one or both margins incurved forming a long fold or channel, 100–400 µm long; costa 75–100 µm wide at base, 45–60 µm wide at mid-leaf, with two rows of ventral cells; basal leaf cells hyaline, rectangular to polygonal, thin-walled, rarely thickened, (16–)25–90 x 8–20 µm; basal marginal cells often quadrate, thick-walled, 12–28(–36) x 12–25 µm; middle and upper cells ovate to isodiametric, (7–)10–15(–25) x 8–13 µm, with 1–2(–4) simple or branched papillae, up to 15 µm long. *Brood bodies* frequent on leaves, more rarely on rhizoids; those on leaf lamina cylindrical to claviform, with brown cell walls, (50–)75–175(–250) µm long, rarely up to 600 µm long and branched; those at apices of some narrow rhizoids fusiform to claviform, (50–)75–150 µm long.

Cladautoicous; perigonia terminal or lateral on male branches, perigonial leaves ovate to elliptical, with rhomboidal cells, costa short; *perichaetia* terminal, perichaetial leaves not different from vegetative ones. *Vaginula* (0.6–)0.75–1.2(–1.5) mm long, cylindrical, naked (although sometimes with some short axillary hairs at base). *Seta* (0.8–)1.0–1.6 mm long, yellowish, twisted anticlockwise when dry. *Capsule* 1.5–2.1 mm long, immersed to emergent, brown-yellowish, fusiform when moist, cylindrical when dry and full of spores, strongly constricted from mouth to the lower third when dry and empty, sharply ribbed, neck gradually tapering to seta, sometimes twisted. Exothecial cells quadrate to shortly rectangular, thin-walled, 35–80 x 12–28 µm, differentiated into 8 exothecial bands 4 cells wide; cells of the bands with darker and thicker walls, 20–85 x 10–25 µm. *Stomata* cryptopore, almost completely covered by surrounding cells, located in the lower half of the urn, usually not reaching the neck. *Operculum* almost plane, rostrate, brownish, with a very thin darker basal ring. *Peristome* double. *Exostome* of 8 triangular, non-splitting teeth pairs, (150–)190–270 µm long, recurved when dry; external surface (Outer Peristomial Layer, OPL) roughly ornamented by thick papillae, occasionally with vermicular lines that are horizontal below and longitudinal above; internal surface (Primary Peristomial Layer, PPL) smooth. *Endostome* of 8 segments shorter than teeth, 100–170(–200) µm long, linear with a wide base, with transversal thickenings; outer side (PPL) smooth; inner side (Inner Peristomial Layer, IPL) smooth or slightly papillose. Connective membrane continuous, smooth or slightly papillose, low. *Calyptra* fusiform when young, when mature campanulate, plicate, with mostly short and inconspicuous, uniseriate, somewhat papillose hairs 50–100(–275) µm long. *Spores* papillose, (9–)10–15(–17.5) µm.

Etymology. The specific epithet ('very hairy') refers to the unique development of the axillary hairs shown by this moss.

Distribution and Ecology. *Orthotrichum pilosissimum* has been found in arid areas from five different counties of the state of Nevada (Fig. IV.3.28) spanning from 1530 to 2350 m of altitude. Until additional records allow drawing a more comprehensive ecological characterization of the species, available data suggest that it seems to prefer narrow streambeds or creek riversides where the severe drought conditions are slightly buffered. It colonizes a wide range of substrates, since it has been found on several types of rocks (volcanic, granitic and slates), and more rarely on roots and trunks of *Populus balsamifera* L. The moss was growing abundantly in most of the localities and seems to be common in suitable areas. It likely occurs in other areas of the state of Nevada and adjacent areas, especially in Eastern California, since some records were found very near the interstate border.



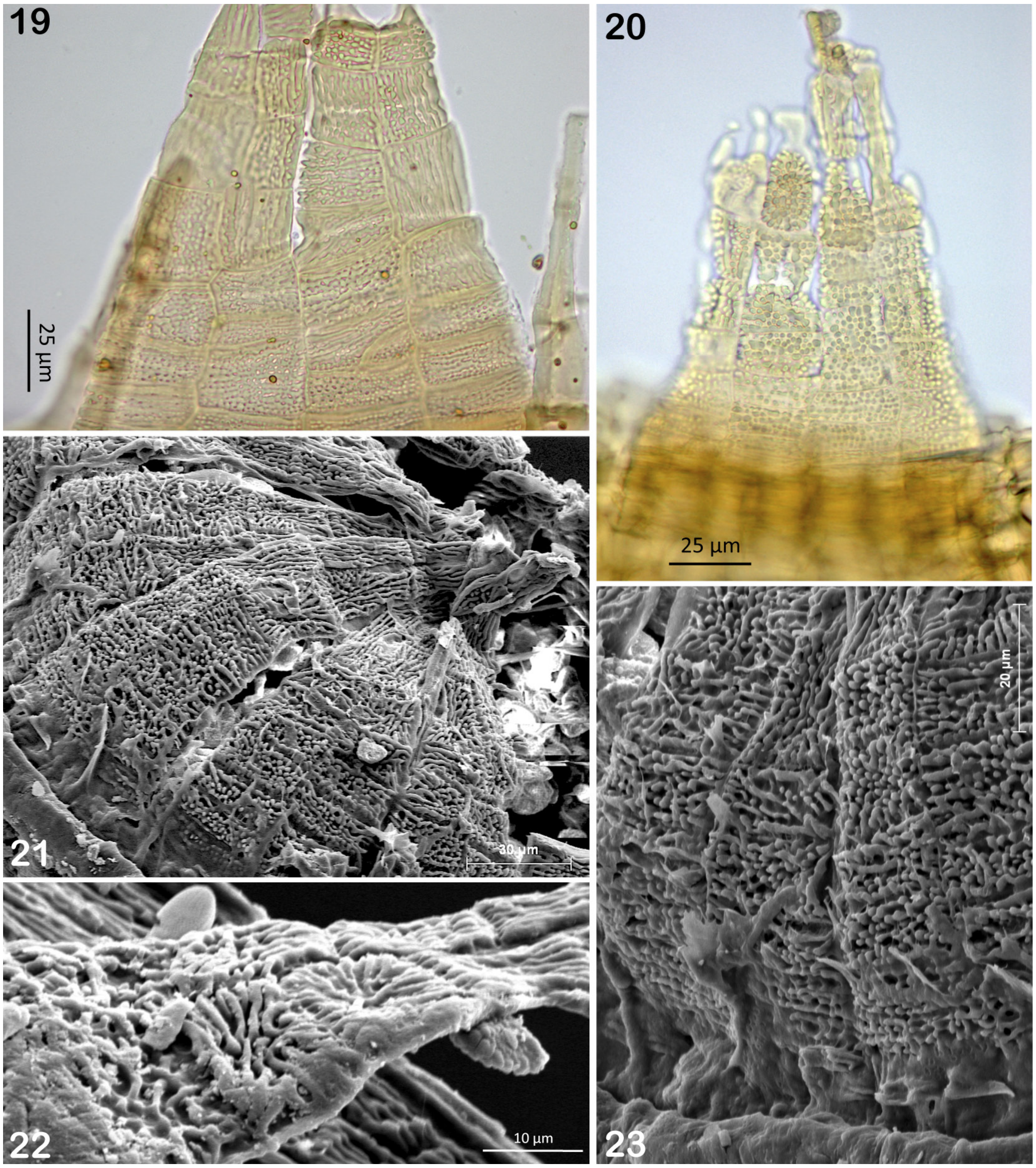
FIGURES IV.3.15–18. *Orthotrichum pilosissimum*. 15. General view of a capsule; note the strongly marked exothecial ribs. 16. Detail of capsule mouth and upper third of the urn showing the peristome configuration when dry. 17. Stoma. 18. View of two endostome segments and one exostome tooth where the completely smooth inner side (exostome PPL) of the tooth can be noticed. (All from holotype).

Discussion

Orthotrichum pilosissimum is easily recognized by its distinctive long and branched axillary hairs that can be detected even in the field with a hand lens, as some of these hairs are so long that they protrude from the foliose shoots and have a white shiny appearance. The hairs are more easily seen if some leaves are removed (Figs. IV.3.2–4). A closer examination under the light microscope shows that fascicles of axillary hairs cover the surface of the stems and leaf axils (Fig. IV.3.5). These hairs are often multiseriate and branched, especially at the base (Fig. IV.3.6).

Axillary hairs are filaments originating from the stem epidermis, especially at leaf insertions; they typically consist of a small row of elongated hyaline cells often subtended by one or two brown cells (Goffinet et al., 2009). In all other species of the genus *Orthotrichum* axillary hairs are unbranched, filiform or slightly club-shaped at the end, about 50–150 µm long and formed by 2–8 cells, plus one or two brown basal cells. Therefore, they are very similar to those of other acrocarpous mosses (see, for instance, Zolotov & Ignatov 2001). Long, filiform axillary hairs exceeding 1000 µm long have been found in Encalyptaceae and Timmiaceae (Horton 1982; Zolotov & Ignatov 2001) but they were always uniseriate and unbranched. Very long, multiseriate axillary hairs have been reported in a sample of *Didymodon tophaceus* (Brid.) Lisa from Arkansas, but they were restricted to a single specimen and thus considered to have a teratological origin (Allen 1992). Finally, densely branched axillary hairs have been reported for some Meteoriaceae species (Quandt et al., 2004) but they are short-celled and do not form long fascicles. Thus, as far as we know, the type of hairs found in *O. pilossisimum* is not only unique within the genus but also among mosses. The distinctiveness of this feature and its uniqueness raise the question whether these hairs have a specific function.

It is widely accepted that the function of axillary hairs is the secretion of a mucilage that prevents the desiccation of the delicate young tips of shoots (Schofield & Hébant 1984), as they are found frequently in the axils of young leaves (although they can also remain in older ones too). However, Hedenäs (1989) suggested that, at least in many modern pleurocarpous mosses, these hairs may have another function or lack any purpose at all, since no correlation was found between the development of these structures and the ecology of different pleurocarpous lineages. Many *Orthotrichum* species grow in environments subject to drought or high insolation, being able to thrive as epiphytes in remarkably dry conditions. Nevertheless, the localities of *O. pilosissimum* are considerably drier than the standards for this genus. It would be reasonable to think that the presence of these unique axillary hairs just in such a xerotolerant species may not be a coincidence and that this peculiar trait has an adaptive significance. The tomentum of axillary hairs covers densely the stem, increasing the contact surface and thus it could play an



FIGURES IV.3.19–23. *Orthotrichum pilosissimum*. 19–20. Structure and ornamentation of exostome teeth in light microscopy (LM); note the high variability of the pattern, that in the basal part of tooth consists in a low, smooth or papillose, reticulum, while in the upper part it has papillae and lines, also smooth or papillose. 21. General view of exostome OPL under SEM. 22. Detail of the upper part of a tooth with a particular ornamentation with lines radially disposed. 23. Image of a papillose basal reticulum. (19 from *Shevock 22294*; 20 from *Shevock 22368*; 21–23 from holotype).

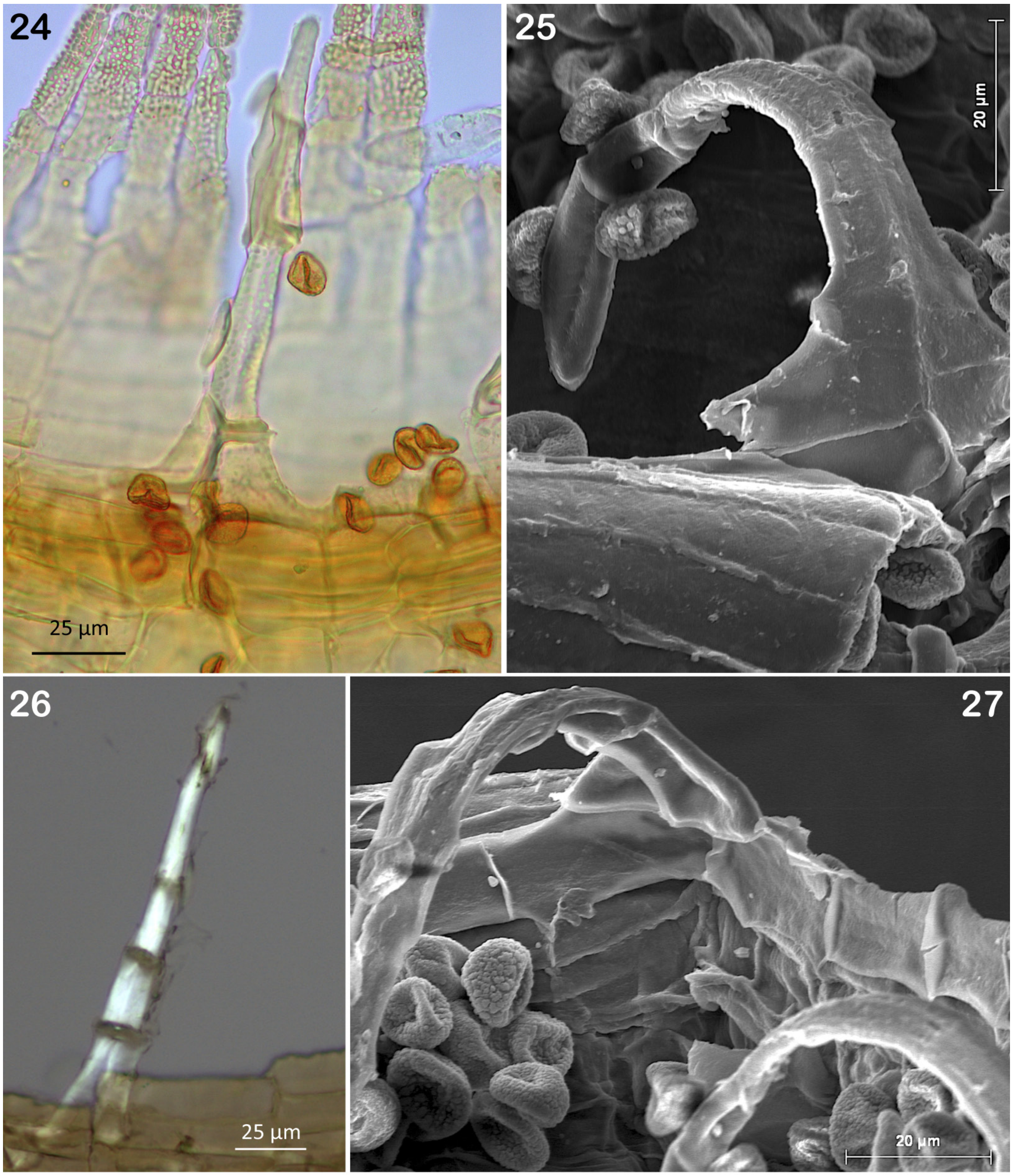
important role in water balance in three different ways: 1) they may contribute to the movement of water along the stem surface, as the paraphyllia supposedly do (Vanderpoorten & Goffinet 2009), 2) they could help to retain extracapillary water for a longer time (Proctor 2000), and 3) they could, since they sometimes protrude above the leaves, enhance the condensation of the atmospheric moisture.

Considering the configuration of its peristome and the cryptopore stomata, *Orthotrichum pilosissimum* is placed in subgenus *Pulchella* (Schimp.) Vitt (Lewinsky 1993).

The stomata location at the lower part of the capsule, the development of leaf brood bodies and the frequently channeled leaf tips make this species similar to *O. tenellum*, *O. norrisii* F. Lara, R. Medina & Garilleti, and *O. bartramii* R. S. Williams. However, even if axillary hairs are not considered, *O. pilosissimum* can still be easily distinguished from these taxa by its leaf shape, distinctly narrowed with strongly revolute margins in the upper half and acuminate to subulate apex, while the other species have broader leaves with blunter apices. In addition, *O. pilosissimum* 1) is cladautoicous, 2) bears calyptrae with inconspicuous hairs, 3) has capsules remarkably constricted in the upper two thirds when dry and empty, 4) has relatively long setae, and 5) has a coarsely papillose ornamentation on the exostome teeth, sometimes with thick lines. *Orthotrichum tenellum*, *O. norrisii* and *O. bartramii* are goniautoicous, have conspicuously hairy calyptrae, capsules cylindrical or only constricted below the mouth, setae shorter than 1.0 mm, and finely papillose exostome teeth. Finally, rhizoidal brood bodies, although not very abundant in *O. pilosissimum* specimens, have never been described in the former three taxa. From the ecological point of view, none of the mentioned species is likely sympatric with *O. pilosissimum*, since the latter colonizes specific enclaves in very dry or desert-like landscapes, while the other species are mostly epiphytes in wetter woodland to forest ecosystems.

Additional specimens studied

U.S.A. NEVADA: Humboldt Co., along stream, Buffalo Creek Canyon about four miles east of Hwy 95, Santa Rosa Range, Humboldt National Forest, Sec. 31, T42N, R38E, elev. ca. 6400–7000 ft., on moist, diffusely lit base of cottonwood, Jun. 1973, *Norris 24481*, *van Horn & Jackson* (UC); Mineral Co., Anchorite Hills, Toiyabe Nat. Forest, Box Canyon, 38º13'31" N 118º39'11" W, 2150 m, on granitic rocks in the creek, Oct. 2008, *Lara, Garilleti, Shevock & Albertos* (herbarium of Univ. Autónoma de Madrid, CAS, UV); Esmeralda Co., Morris Creek, north base of Boundary Peak, 37º52'33" N 118º23'37" W, elev. 6800 ft., granitic boulders along creek, riparian area of rose, willow and cottonwood, on rock wall above creek, May 2002, *Shevock 22294* (CAS 1039519); Nye Co., Alta Toquima Wilderness, Pine Creek above Pine Creek Campground, 38º47'36" N 116º51'29" W, 7700 ft., riparian woodland with quaking aspen and black cottonwood within a pinyon pine-Utah juniper woodland, on volcanic rock wall, *Shevock 22397 & Glazer*, May 2002 (CAS 1040002); Death Valley National Park, Grapevine Mts., Amargosa Range, on rocky slopes just south of Strozzi Ranch site and Brier Spring, 36º56'14" N 117º04'37" W, 6400 ft., among volcanic mudstone outcrops within a pinyon pine-Utah juniper woodland, Dec. 2001, *Shevock 21581, Davis & Davis* (UC 1754355); Eureka Co., along 004, 9.4 miles east of Monitor Valley via, road 425, 39º18'04" N 116º31'01" W, elev. 7500 ft., large volcanic boulders and outcrops in a pinyon pine and juniper woodland with sagebrush, on rock wall, May 2002, *Shevock 22368 & Glazer* (CAS 1039515).



FIGURES IV.3.24-27. *Orthotrichum pilosissimum*. 24. LM view of an endostome segment showing a light papillose ornamentation on its inner side (IPL), also seen in the connective membrane at right. 25. General view of an endostome segment under SEM. 26. A segment as seen with polarised light; the intense bright suggests a very organized disposition of cellulose molecules, most probably in the IPL better than in the PPL, since the exostome teeth PPL remains dark. 27. Partial view of a segment with a well-developed connective membrane; in this specimen both structures are smooth. (24 from *Shevock 22368*; 25, 27 from holotype; 26 from *Shevock 21581*).

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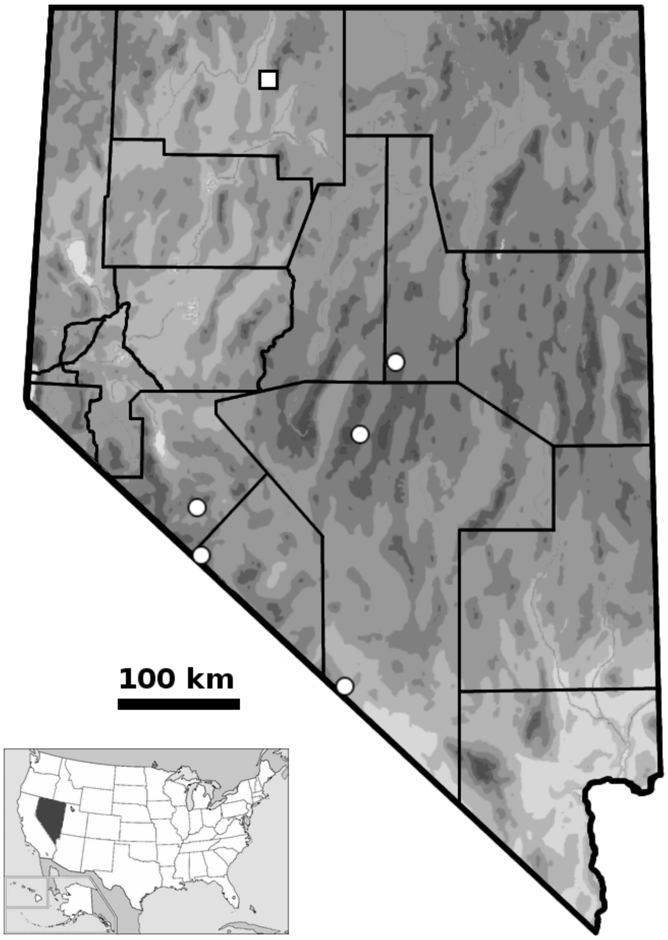

Figure IV.3.28. Known distribution of *Orthotrichum pilosissimum*. The locus classicus is identified by a square.

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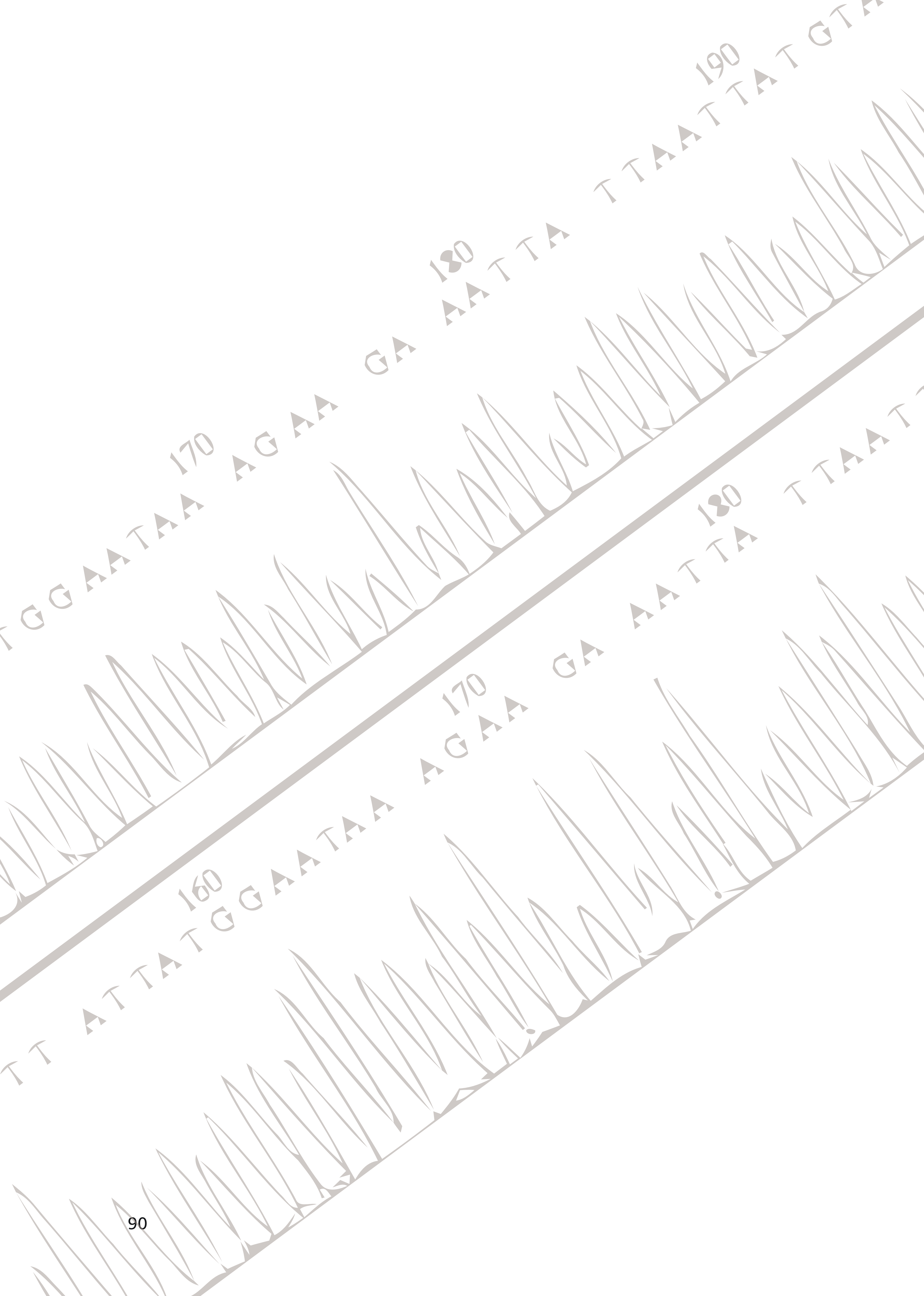
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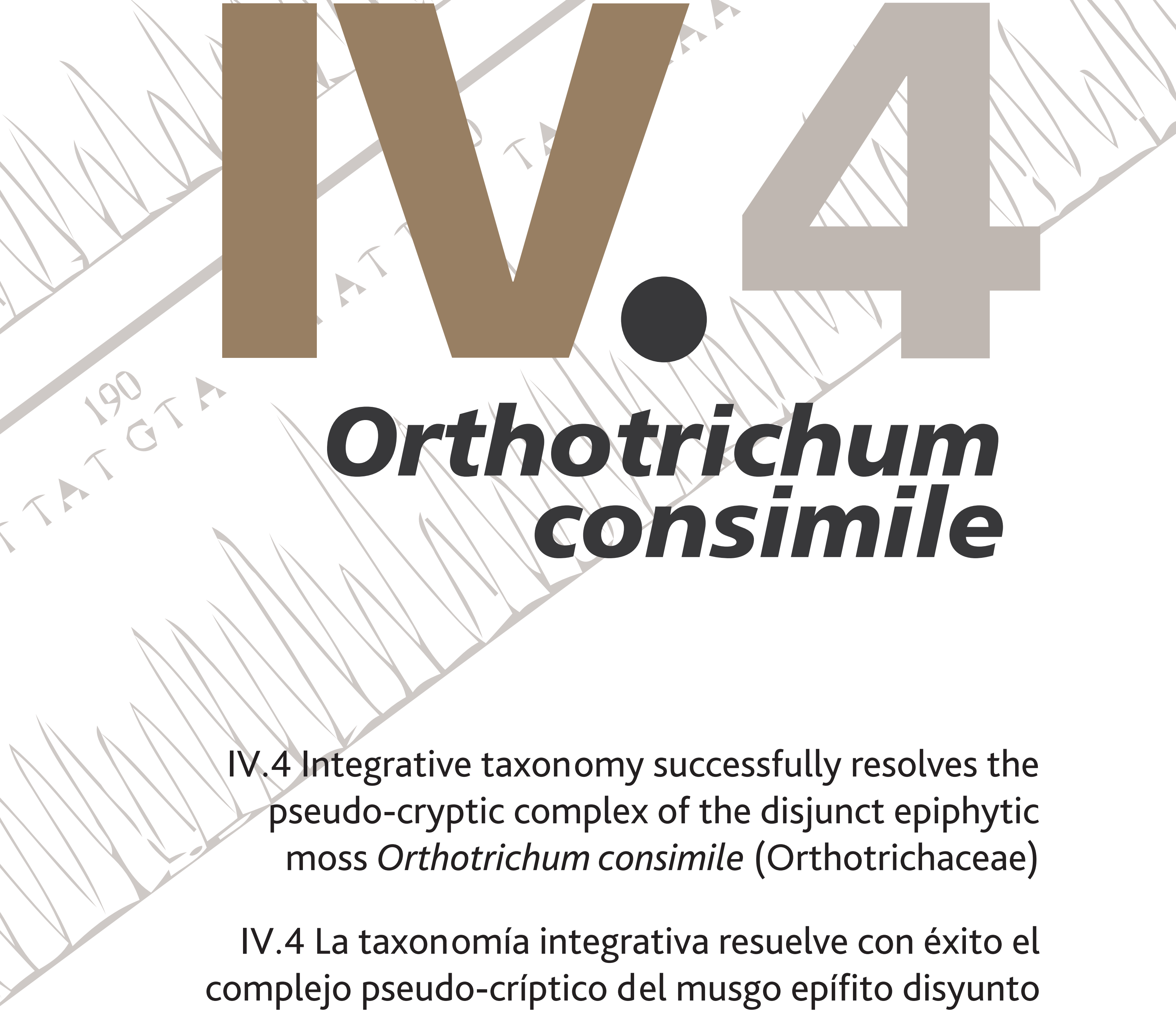

IV.4

Orthotrichum consimile

IV.4 Integrative taxonomy successfully resolves the pseudo-cryptic complex of the disjunct epiphytic moss *Orthotrichum consimile* (Orthotrichaceae)

IV.4 La taxonomía integrativa resuelve con éxito el complejo pseudo-críptico del musgo epífito disyunto *Orthotrichum consimile* (Orthotrichaceae)

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Enviado a *Taxon*

Abstract

Orthotrichum consimile Mitt. is an epiphytic moss known from highly oceanic forests, supposedly distributed in Western North America and Western Europe. Its wide morphological variation and complex taxonomic history suggest that it may be a complex of cryptic species rather than a single taxon. This hypothesis has been tested by means of integrated morphological and phylogenetic investigations. We have studied the morphology of both herbarium specimens and fresh collections assigned to *O. consimile* and allocated them to three morphological groups. We tested the monophyly of these putative taxa based on standard phylogenetic inferences from four chloroplast loci. These inferences validated two of the groups but split the third one into two further clades. This prompted us to re-assess the characters of the lineages and successfully described them morphologically. We show that *O. consimile* s.l. is in fact an assemblage of four independent lineages, which can be defined unambiguously on morphological characters: *Orthotrichum consimile* s.s., *O. columbicum* Mitt. (resurrected from synonymy), *O. confusum* sp. nov. and *O. persimile* sp. nov. Except for *O.columbicum*, which has a transatlantic distribution, these species are endemic to Western North America. Phylogenetic inferences increasingly resolve cases of so-called cryptic speciation within bryophytes exhibiting broad geographic distributions. In most such cases the hypothesis of cryptic differentiation has not been tested. Here we show that complementary morphological and phylogenetic studies can lead to the resolution of an apparently cryptic complex and the discovery of new, morphologically defined, species.

Resumen

Orthotrichum consimile Mitt. es un musgo epífito que habita bosques oceánicos, supuestamente distribuido en el oeste de Norteamérica y Europa occidental. Una amplia variabilidad morfológica y su compleja historia taxonómica sugieren que podría tratarse en realidad de un complejo de especies crípticas en lugar de un único taxón. Esta hipótesis se ha testado de forma integrativa mediante estudios morfológicos y filogenéticos. Se han examinado materiales de herbario y muestras de reciente recolección identificadas como *O. consimile* y se les ha clasificado en tres grupos morfológicos. El carácter monofilético de estos taxones hipotéticos se estudió mediante una reconstrucción filogenética a partir de cuatro regiones del genoma cloroplástico. Dos de los grupos quedaron validados, mientras que el tercero fue a su vez dividido en dos clados. Esto motivó una re-evaluación de los caracteres que permitió la circunscripción morfológica de dichos linajes. Se muestra así que *O. consimile* s.l. está en realidad constituido por un conjunto de cuatro linajes independientes que pueden definirse inequívocamente mediante caracteres morfológicos: *Orthotrichum consimile* s.s., *O. columbicum* Mitt. (recuperado de la sinonimia), *O. confusum* sp. nov. y *O. persimile* sp. nov. Excepto *O. columbicum*, que presenta una distribución transatlántica, estas especies son endémicas del oeste de Norteamérica. Las reconstrucciones filogenéticas resuelven con frecuencia casos considerados de especiación críptica entre briófitos con amplias distribuciones geográficas. En la mayoría de ellos no se llega a comprobar si se trata realmente de especies crípticas. Aquí se muestra que los estudios morfológicos y filogenéticos realizados de forma complementaria pueden conducir a la resolución de grupos aparentemente crípticos y al descubrimiento de nuevas especies, definidas morfológicamente.

Introduction

Cryptic species and their delimitation constitute a major challenge to the assessment of global biodiversity, the accurate modeling of species distribution and the development of adequate conservation strategies (Bickford et al., 2007). Phylogenetic inferences increasingly highlight robust structuring within morphologically defined taxa, yet how this hidden diversity should be considered remains controversial (Trontelj & Fišer, 2009). In part, the controversy around the taxonomic inflation that may accommodate the phylogenetic hypotheses (Isaac et al., 2004; Padial & De la Riva, 2006) resides on the practicality of diagnosing the cryptic species, a problem particularly acute in morphologically austere lineages (Bickford et al., 2007), such as bryophytes. Organisms with a simple structural organization exhibit few morphological traits, and discontinuity in the variation of these traits is likely more difficult to identify. Moreover, robust phylogenetic structuring of the genetic diversity is often uncovered within taxa that are morphologically rather stenotypic (Klautau et al., 1999; del Carmen Molina et al., 2004; Otalora et al., 2010), suggesting that reliance on morphological traits alone may severely underestimate the real taxonomic richness in non vascular plant lineages, such as mosses.

Bryophyte diversity is estimated in about 17,000 extant species (Vanderpoorten & Goffinet, 2009). Many mosses occur over large geographic areas spanning across continents and oceans (Frahm, 2009; Schofield & Crum, 1972). Species with disjunct distribution are the prime target for phylogeographic studies and which almost invariably conclude on the presence of cryptic species (see Shaw, 2001 and Heinrichs et al., 2009 for a review).

The processes that underlie those large ranges are of very different nature. They may result from the fragmentation of a broad ancient distribution (e.g. Heinrichs et al., 2006; McDaniel & Shaw, 2003) or from long distance dispersal of spores (e.g. Feldberg et al., 2007; Vanderpoorten et al., 2008). However, phylogenetic structure and evidence of cryptic speciation has also been uncovered among sympatric populations (Fernandez et al., 2006; Wachowiak et al., 2007). Although this kind of contributions reveals a hidden diversity, most of them lack a morphological reevaluation of the specimens and a taxonomical integration of the results, (with noteworthy exceptions: e.g. Renner et al., 2010), making this diversity also unattainable. This makes the assessment of the bryological diversity difficult or impossible for scientists without access to molecular methodologies.

The subject of this work is an epiphytic moss, *Orthotrichum consimile*, which belongs to one of the most diverse moss genera (more than 150 species), but with many of its insights still scarcely understood. So far, O. consimile is thought to be present in the highly oceanic temperate forests of both Western Europe and Western North America. This is not an uncommon disjunction among Holartic bryophyte species (Schofield, 1988). Besides this distinct distribution, this taxon has an intricate taxonomical and nomenclatural history, including a number of misinterpretations and synonyms, and thus constitutes a well-suited taxon to test species delimitation. As a part of our taxonomic revision of subgenus *Pulchella* (Schimp.) Vitt in North America, we detected a wide morphological variability among specimens identified as *O. consimile* that suggested a previously overlooked hidden diversity. In order to resolve this complex, we have studied it from an integrative taxonomy perspective (Dayrat, 2005), seeking the “reciprocal illumination” (Steele & Pires, 2011) between a careful taxonomical revision and a molecular phylogenetic reconstruction.

Material and methods

Taxon sampling and morphological studies. We studied 134 specimens, both fresh samples from recent collection campaigns in California, Oregon, Washington, and British Columbia, and herbarium materials (including types) from B, CAS, COLO, FH, MICH, NY, UC and Authors' herbarium at Universidad Autónoma de Madrid.

The taxonomy of the genus *Orthotrichum* Hedw. is arguably very complex. Many of the diagnostic characters used for this revision are discussed in Lewinsky (1993), while others are the result of the previous experience of our research group. More than a hundred characters can be examined in *Orthotrichum* taxonomy, although only some of them show variability within the restricted ingroup of this study. Most relevant characters include leaf shape, leaf margin curvature and leaf apices differentiation, vaginula length and hairiness, calyptra hairiness, seta length in relation to the urn, stomata position, number of cell rows forming the exothecial bands, peristome configuration, teeth and segments ornamentation and spore size and ornamentation.

For the phylogenetic reconstruction we selected a subset of recent samples that were representative of the morphological diversity and its geographic distribution. Several additional specimens belonging to other *Orthotrichum* species with cryptopore stomata were also included in the selection, with special emphasis on taxa similar to *O. consimile* or with a significant presence on the Western coast of North America. Other Orthotrichaceae were used as outgroup bringing the total sampling to 56 exemplars. The identity of all the specimens used for DNA extraction was carefully confirmed. Specimens' data such as localities, herbarium and GenBank accession numbers are listed in the Appendix.

DNA extraction and sequencing. Genomic DNA was extracted from fresh or recent herbarium material following the CTAB extraction protocol described in Doyle & Doyle (1987) with some modifications which had already provided good results with Orthotrichaceae (Goffinet et al., 1998). Alternatively, the DNA of some particular specimens was extracted with the DNeasy Plant Mini commercial kit (Qiagen, California) following the manufacturer's instructions. Four chloroplast loci were selected for amplification and sequencing: *atpB-rbcL, rps4, trnG and trnL-F*. All of them have been used successfully for bryophyte phylogenetic reconstruction in the past (Stech & Quandt, 2010). The primer pairs used for each locus were *atb1/rbcL1* (Chiang et al., 1998), *rpsA/trnaS* (Nadot et al., 1994; Souza-Chies et al., 1997), *trnGF/trnGR* (Pacak & Szweykowska-Kulinska, 2000) and *trnC/trnF* (Taberlet et al., 1991).

Amplifications by PCR were performed in a final volume of 25 µL with 0.2 µL Taq DNA Polymerase (for *loci atpB-rbcL and trnG*) or HotMaster Taq DNA Polymerase (for *loci* *rps4 and trnL-F*) 5 PRIME (Hamburg, Germany), 2.5 µL of the Mg2+ Buffer provided by the manufacturer, 1 µL of 10µM dNTP mix, 1 µL of each primer (10 µM) and 1µL of the DNA extract. After a initial denaturation step of 5 min at 94ºC, 30 cycles were carried out consisting of 30 s denaturation at 95ºC, 1 min of annealing (48ºC for *trnG* and 52ºC for the other *loci*) and a 30 s extension at 68ºC, followed by a final extension step of 7 min. PCR products were purified using the Nucleospin Extraction Kit (Machery-Nagel, Düren, Germany). Sequencing reactions were conducted in a final volume of 10 µL with ABI BigDye Terminator and cleaned as described in Buck et al. (Buck et al., 2000). The samples were read using an ABI Prism 3100 Genetic Analyser (Applied Biosystems, Foster City, California, USA) with the same primers used in the PCR reactions.

Phylogenetic analyses. Sequence contigs were assembled using Sequencher 3.1.1. (Machery-Nagel) or Geneious 5.1.7.0 (Biomatters Ltd.) and the matrices for each locus were built using PhyDE® (Müller et al., 2006). The sequences were first aligned with Clustal W (Larkin et al., 2007) or MUSCLE (Edgar, 2004), then adjusted manually and trimmed at the ends.

The substitution model for each matrix locus was inferred using jModelTest 0.1.1 (Posada, 2008). The models were selected according to the Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC). If the two criteria did not agree in the best choice, the model that showed a higher likelihood (*lnL*) for both criteria was chosen. Additional replicates of the analyses with the alternative model choice were conducted to check that there were no substantial changes in the results. Indels of the full alignment were coded as an adjacent block according to the simple-coding algorithm (Simmons & Ochoterena, 2000) implemented in SeqState 1.4 (Muller, 2005). In order to test the congruence between the different sets of molecular data, we performed the ILD test (Farris & al., 1994) based on 1,000 replications with TBR selected as swapping algorithm.

Phylogenetic analyses were performed using three methods: Maximum Parsimony (MP), Maximum Likelihood (ML) and Bayesian Inference (BI). Codified indels were included in the MP matrix (not partitioned), while a 5-partition matrix was used for the ML and BI: one partition for each locus plus a fifth partition for the coded indel block. For ML and BI analyses, a substitution model was applied to each partition. Models were inferred from jModelTest, except for the indel block to which the F81 model was applied as recommended in Ronquist et al. (2005). MP analysis was performed using MEGA 5 (Tamura et al., 2011) inferring for the optimal trees by the Close-Neighbor-Interchange Method (CNI) on random trees and the support of the nodes was estimated from the consensus of trees obtained from 1000 heuristic bootstrap pseudoreplicates (Felsenstein, 1985). ML analysis was conducted using GARLI 2.0 (Zwickl, 2006). The branch supports of the tree with the highest likelihood score were estimated based on the consensus of 200 bootstrap pseudoreplicates. The phylogenetic reconstruction by BI was obtained using

MrBayes 3.1.2 (Ronquist & Huelsenbeck, 2003).The Markov Chain Montecarlo (MCMC) simulation was run for 2,000,000 generations with two runs and four chains, sampling trees and parameters each 1,000 generations. After checking that the stationarity had been reached and burning the 25% of the initial sampled trees, a 50% Majority-rule consensus tree was built and plotted using FigTree 1.3.1 (Rambaut & Drummond, 2010).

The species delimitation was additionally tested using the genealogical sorting index (gsi_T; Cummings et al., 2008). For a given rooted phylogeny, this method estimates the degree of exclusive ancestry of the groups manually labeled, in this case, according to the morphological hypothetic delimitation. The gsi_T maximum value is 1, acting as a monophyly test, while the null hypothesis to be rejected is that the labeled clusters are random. Coalescent simulations for 1,000 generations were used for the resulting collection of bootstrap pseudoreplicates trees for (ML) and postburn-in trees (IB). The method also provides a P-value for the probability that a random mating could result into the same clustering.

Results

Preliminary species delimitation. The first morphological approach resulted in the detection of three different morphotypes, preliminarily called A, B and C. These morphotypes shared a set of conspicuous characteristics that can explain why they had been long overlooked: they grow forming distinct tufts, their leaves are variably flexuose when dry, and the capsules are more or less exserted, furrowed and with well-developed peristomes. In addition, all these morphotypes are superficially similar to other Orhotrichaceae that share the habitat in the same areas, chiefly *Orthotrichum pulchellum* Brunt. and several species of the genus *Ulota* Mohr. So, the correct identification of *O. consimile* s.l. from these other epiphytic mosses is itself already a difficult task. Besides, sometimes several of these morphotypes appear mixed in the collections, making the discrimination even more complicated.

Despite their mutual resemblance, the three morphotypes could be accurately distinguished by a distinct combination of characters (Table IV.4.1): Morphotype A is differentiable by its endostome of 16 segments, exostome teeth with striate inner surface and usually an evident, long seta. Morphotype B is the only one with leaves that have plane margins, clearly hairy vaginula and stomata placed very low in the urn and the neck. On its part, Morphotype C can be mainly discriminated by its filiform to unguiculate leaf apices, stomata mostly situated in middle urn and distinctly radiculose stems. In the context of current *Orthotrichum* taxonomy, such morphological differentiation would suffice to recognize these morphotypes as distinct species.

	Morphotype A *Orthotrichum columbicum*	Morphotype B *Orthotrichum persimile*	Morphotype C	
			Morphotype C1 *Orthotrichum consimile*	Morphotype C2 *Orthotrichum confusum*
Rhizoids position	Lower parts, somewhat ascending along stem	Restricted to the lower parts	Distinctly ascending along stems	
Rhizoids surface	Mostly smooth	Rough	Mostly smooth	
Leaf margin	Recurved	Plane on one or both margins	Recurved	
Leaf apex	Acuminate, frequently chanelled	Acute or subacute, ending in a large hyaline cell	Typically ending in a filiform or unguiculate acumen; sometimes channeled	
Vaginula length and shape	(0.4–)0.5–0.8(–1.2) mm. Cylindrical	0.5–0.75 mm. Cylindrical	(0.25–)0.4–0.8 mm. Cylindrical to frustum-shaped	(0.6–)0.8–1.1(–1.3) mm. Cylindrical or doliiform
Vaginula hairiness	Naked	Hairy	Naked or with sparse, stout hairs	Naked
Calyptra hairiness	Naked	Hairy (1–2 seriate, mostly smooth hairs)	Variably hairy (stout, papillose hairs)	Naked or almost so
Seta length	(2.0–)2.5–5.5 mm	1.2–1.7 mm	(0.8–)1.2-2.0(–4.0) mm	1.1–1.8(–2.3) mm
Stomata position	Middle and lower half of the urn, not on the neck	Lower half of the urn and neck	Middle urn or lower half, but absent from the neck	
Inner exostome surface (PPL)	Clearly striated	Smooth or almost so	Smooth or almost so	
Endostome configuration	Commonly 16 segments, sometimes the intermediate reduced	8 segments	8 segments	
Spore size	(12–)14–17(–20) µm	15–17(–20) µm	8–13(–17) µm	12–16(–20) µm

Table IV.4.1. Morphological characters segregating the different morphotypes detected in this work and the subsequent species delimitation.

The formal naming for *Orthotrichum consimile* and related taxa has been always a delicate matter, as a consequence their complexity. In his revision of the genus *Orthotrichum* in North America North of Mexico, Vitt (1973) perfectly summarizes this by listing the known 13 synonyms and combinations of his concept of *O. consimile*, including 7 heterotypic synonyms. In order to find out which correct names correspond to the observed species, we studied the available type materials of those names that could correspond with the recognized morphotypes.

Morphotype C corresponds to the lectotype of *O. consimile* (NY!), and no other type material of the different synonyms under *O. consimile* can be ascribed to this morphotype. Morphotype A matched the description of *O. columbicum*, a species described simultaneously by Mitten (1864). Depending on the author, in the last century this species was considered a variety of *O. pulchellum* (Grout, 1936) or a mere synonym of *O. consimile* (Vitt, 1973). The type specimen of *O. columbicum* could not be found, but according to the protologue and after the study of a rediscovered specimen named by Mitten himself (TR-Venturi!), morphotype A can be unambiguously assigned to this name. Consequently, *O. columbicum* is here reinstated. The type materials of other names, particularly *Orthotrichum winteri* Schimp. (NY!), *O. pulchellum* var. *longipes* Sull. (FH!), *O. pulchellum* var. *productipes* Renauld & Cardot (FH!) and *O. ulotaeforme* Renauld & Cardot (FH!)–and all their subsequent combinations– corresponded also to morphotype A, and therefore to *O. columbicum*. The type material of *Orthotrichum pulchellum* var. *leucodon* Vent. in Röll was lost during a postal return to TR (Garilleti et al., 2007) and its identity could not be checked. Finally, morphotype B was not found among the type specimens of this complex and, as far as we know, it has not been yet named as a distinct species. We describe it here for the first time as *Orthotrichum persimile*. Additionally we found out that the type of *Orthotrichum hendersonii* Renauld & Cardot (FH!), synonymized to *O. consimile* by Grout, is in fact *O. pulchellum*.

Phylogenetic testing of the preliminary delimitation. The combined alignment comprised 2252 nucleotide sites, including 267 informative and 89 autopomorphic characters, complemented by 108 binary characters representing the coded indels. The ILD test did not find significant incongruence among the combination of loci except for atpB-rbcL tested with rps4 and trnG. The results of the individual analysis of each locus were manually checked to confirm that there were no conflicting clades at the species delimitation level.

Regardless of the method, the samples identified as *Orthotrichum columbicum* (morphotype A) and *O. persimile* (morphotype B) are resolved in two well supported clades with gsi_T=1 (Fig. IV.4.1). By contrast, exemplars of *O. consimile* s.s. (morphotype C) compose a polyphyletic assemblage of two robust lineages (C1 and C2; Fig. IV.4.1).

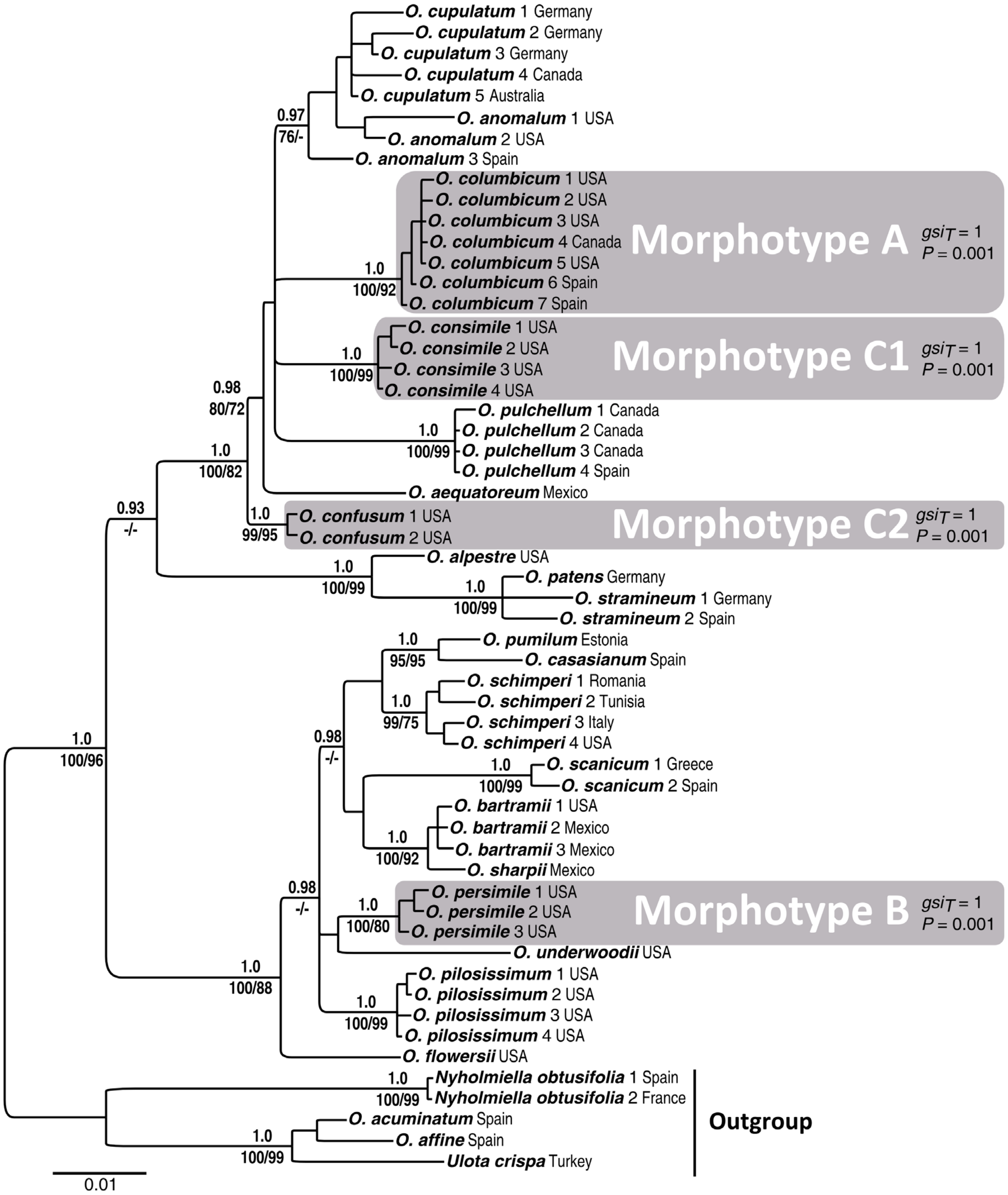

Fig. IV.4.1. Majority-rule consensus tree of the Bayesian Inference obtained from the analysis of the partitioned matrix for the loci *atpB-rbcL* (GTR+G model), *rps4* (GTR+G), *trnG* (HKY+G), *trnL-F* (HKY+G) and indel block (F81) after 2,000,000 generations with two runs and four chains. Trees were sampled each 1,000 generations and 25% of the initial trees were discarded with the burnin setting. Bayesian Posterior Probabilities (PP) are shown over the branches, while bootstrap values for Maximum Likelihood / Maximum Parsimony are displayed below. Support values insufficiently strong (PP<0.9; bootstrap<70) are not shown. Genealogical Sorting Index gsi_T of the species candidates and its P-value are displayed on grey boxes.

Orthotrichum columbicum, clade C1 and *O. pulchellum* share a common ancestor with a clade of several samples belonging to the subgenus *Orthotrichum* (sensu Lewinsky 1993; *O. anomalum* Hedw. and *O. cupulatum* Hoffm. ex Brid.) but the relationships among these lineages are ambiguous, and their affinities to *O. aequatoreum* Mitt., an Andean species, are unresolved. The clade C2 is sister to this large clade and together all these taxa compose a strongly supported unique ancestry. Morphotype B (*Orthotrichum persimile*) is distantly related to this group (Fig. IV.4.1), and resolved among species of *Orthotrichum* with immersed stomata belonging to subgenus *Pulchella* (sensu Lewinsky 1993). The affinities of *O. persimile* to indivudal species within this clade remain ambiguous.

Reevaluation of morphotype C. The split of morphotype C into two clades suggested the presence of two cryptic taxa that could not be distinguished by morphology. In order to assess this, a careful reevaluation of the specimens used for the phylogenetic analyses was undertaken. We found some key features discriminating both morphotypes (Table IV.4.1), although they are rather subtle and easily misinterpreted, due to their intrinsic variability. As a result, by using this updated characters assessment, all the samples of morphotype C could be ascribed either to C1 or C2. Morphotype C1 shows hairy calyptrae, comparatively shorter vaginulae, and smaller spores, while morphotype C2 has long vaginulae and commonly, naked calyptrae and larger spores. The identification of *O. consimile* was clear since the protologue of this species refers to a moss with hairy caliptra and coincidently the type specimen exactly matched specimens of morphotype C1. As for the samples ascribed to morphotype C2, it turned out that they belong to an unnamed species, here described as *Orthotrichum confusum*.

Discussion

Taxonomical history. This work shows that the current concept of *Orthotrichum consimile* represents indeed a morphologically and phylogenetically heterogeneous assemblage of four different species, whose segregation is independently and complementarily supported by morphological examination and molecular phylogenetic analysis. The morphological resemblance among the members of the studied complex was the cause of the confusion and misinterpretations that can be tracked back for more than a century. The existence of four different, diagnosable but tricky species, two of them originally described in 1864 (*O. consimile* and *O. columbicum*) and two so far overlooked (*O. persimile* and *O. confusum*), has lead to the idea of a single taxon with very wide variability ranges.

Orthotrichum consimile s.s. is a rather scarce and inconspicuous species that has been frequently confused with *O. columbicum*. The latter is a much more frequent, abundant, robust and, therefore, eye-catching moss. Consequently, after a long taxonomical history, far from free of misinterpretations, *O. columbicum* became progressively the generally accepted idea of *O. consimile*. Both species were described simultaneously by Mitten (1864) from materials collected by Lyall in Vancouver Island. Mitten himself remarks in the protologue of *O. consimile* the similarity between the two taxa. The only diagnostic difference he points out for distinguishing both species is the leaf size. However it can be inferred from the original descriptions that *O. consimile* has a hairy calyptra and ovate-lanceolate leaves while *O. columbicum* has a naked calyptra and long lanceolate leaves. This is congruent with our morphological revision, but Mitten does not mention some other relevant differences, like the 16-segment endostome or the more clearly exserted capsule of *O. columbicum*. Materials of *O. columbicum* named by Mitten and found in TR-Venturi exhibit consistently all these traits.

Problems with the *O. consimile* group grew up during the last quarter of 19th century, with the addition of several new names for the same species (see synonyms under *O. columbicum* in the species description section), mainly from Western North America. In Europe, two years after the formal description of *O. consimile* and *O. columbicum*, a population of the latter was discovered in Germany, and Schimper (1866) published it as *Orthotrichum winteri* Schimp. (B!). This author emphasized the similarity with *O. pulchellum* but he apparently was not aware of Mitten's work. Different European bryologists argued the specific status of *O. winteri* in relation to *O. pulchellum* (Lewinsky-Haapasaari et al., 1995). Grout (1936) first suggested the identity between the European *O. winteri* and the North American *O. consimile*. He also propounded the synonymization of seven American taxa with *O. consimile*. However, Grout's work generated an important confusion by proposing the combination of the name *O. pulchellum* var. *columbicum*, and at the same time making a description of *O. consimile* that mixed the morphological traits of both *O. consimile* and *O. columbicum*. In the discussion under *O. consimile*, Grout (1936) includes a highly significant phrase: "Although there are wide variations in size, length of seta, and number of segments, and the calyptra may be naked as well as slightly hairy as in the type, I can see but one specific type…". Much later, Lawton (1971) kept this mixed concept of *O. consimile*, while formally considered *O. winteri* and, for the first time, *O. columbicum* among its synonyms. Vitt's modern monograph for North American *Orthotricha* (Vitt, 1973) agreed these concepts, also maintained in the world revision by Lewinsky (1993). The useful characters for the identification of the species of this complex have been already mentioned (Table IV.4.1) and a key to species including *O. pulchellum* is also given before the species description section.

Distribution and ecology. *Orthotrichum consimile* has been largely considered an example of species occurring in western North America and Europe (Vitt, 1973; Lewinsky, 1993). In North America, it has been reported along the Pacific coast from California to Alaska, plus Idaho (Vitt, 2009). Also in the Old Continent has been reported from its western countries, from Portugal and Spain to Great Britain and Germany (Blockeel et al., 2008). However, after the splitting of the complex into four different species the actual distribution of each one is an open question. Our observations, based on herbaria revisions and own collections throughout the two continental areas involved, draw an interesting new panorama. Indeed, the only disjunct species seems to be *Orthotrichum columbicum*. It is also, by far, the most abundant and widespread species of the complex along the Pacific North America. We have found it from Central California to northern British Columbia (Fig. IV.4.10), and probably most records of *O. consimile* from Southern Alaska may correspond to *O. columbicum*. On the other hand, it is the only one in Western Europe, where it is much more scattered and unusual than in America. It is too early to assess the origin of this disjunction. The phylogenetic reconstruction here presented suggest that the European populations of *O. columbicum* may be the basalmost in the clade, although the sampling is very scarce and further research would be needed before reaching any phylogeographic conclusion.

The remaining three species are, as far as we know, North American endemics. *Orthotrichum consimile* is a widespread moss found from the northern third of California to northern British Columbia. It frequently coexists with *O. columbicum*, but it is much less frequent, becoming rare north of Vancouver area (Fig. IV.4.10). It likely reaches the southernmost territory of Alaska. *Orthotrichum confusum* and *O. persimile* seem to have narrower distribution areas and so far they have only been recorded in the northern half of the state of California (Fig. IV.4.10). These two endemics probably inhabit the southern half of Oregon, an area not yet studied by us; however, we have failed to find them during our recent prospections around the Oregon-Washington border and further north. Northern California, an area of special interest for bryophytes in general (Norris, 1997) stands out as the most important area for the group of species here studied. They are all four present there, sharing the same kind of habitats, mostly trunks and branches of trees and shrubs in humid coniferous forests.

Remarks about integrative taxonomy. Finally, it is necessary to emphasize the role of integrative taxonomy to untangle this pseudo-cryptic group of species. This is a good example of how two different approaches (classical taxonomy and molecular phylogenetic reconstruction) become indispensable as they reciprocally "illuminate" each other to assess a robust species delimitation (Steele & Pires, 2011). On one hand, without the phylogenetic studies, the taxonomic revision would have succeeded in the identification of three species, but very probably would have not been able to segregate *Orthotrichum consimile* and *O. confusum* into separate entities. It would have failed also inferring the relationships among the studied taxa, as most likely all the species in the complex would have been regarded as closely related without realizing that *O. persimile* belongs to a separate lineage, i.e. morphologically convergent with the other considered species.

On the other hand, without the contribution of the specialized taxonomy, it is possible to think that the phylogenetic analysis would have detected the four species, but only assuming that the problem were correctly targeted and that a blind selection of samples would had included the four taxa by chance, something highly improbable. The interpretation, however, would have stressed once again that *O. consimile* is just one more example of disjunct bryophyte species with a wide distribution range and a cryptic, complex and intangible genetic diversity that cannot be faced by bryologists without using molecular methods. In brief: this interpretation would have failed providing any taxonomical solutions. With this combined approach, the resolution of the complex is not only more robust and trustworthy than those that could have been achieved separately; it also makes the identification of this previously hidden diversity more accessible and affordable for a wider number of researchers and naturalists.

It becomes more and more evident that cryptic species, whether true or pseudo-cryptic, are a gap in the scientific knowledge that may affect to relevant decisions in fields of understanding, modeling and conserving biodiversity. Although "morphologically austere" lineages are thought to lack characters for a proper taxonomic delimitation (Bickford et al., 2007), this assumption should not be taken for granted and the participation of specialized taxonomists should always be claimed. It is reasonable to think that situations analogous to the case exposed in this work may be widespread among other cryptogamic lineages.

Systematic Section

Key to species. We propose here an artificial key to the *Orthotrichum* species of Western North America similar to *O. consimile*, i.e.: those with cryptopore stomata, exostome teeth recurved when dry, acute leaves more or less flexuose-crisped, and exserted capsules.

1. Exostome reddish, exothecial bands mostly 2 cells wide.....................***O. pulchellum***

1'. Exostome not reddish, exothecial bands mostly 4–6 cell wide.................................**2**

2. Endostome of 16 segments (sometimes the 8 intermediary ones reduced), exostome PPL with conspicuous longitudinal striae, operculum with a wide bright red to crimson basal rim, seta commonly 2.5–5.5 mm long, calyptra totally naked ....***O. columbicum***

2'. Endostome of 8 segments, exostome PPL smooth or almost smooth, operculum with thin orange basal rim, seta commonly 1.0–2.0 mm, calyptra naked or with more or less abundant hairs………………………………………………………………**3**

3. Vaginula clearly hairy, calyptra variably hairy, stomata in the lower half of the urn and neck, leaf margins plane (sometimes one leaf margin variably recurved), leaf apex various, not filiform, rhizoids rough, not noticeably ascending along the stem………………………………………………………………***O. persimile***

3'. Vaginula naked or very sparsely hairy, calyptra naked or hairy, stomata mainly in the middle urn (rarely in the lower half, never on the neck), leaf margins recurved, leaf apex commonly filiform, rhizoids smooth, ascending along the stems………………………………………………………………**4**

4. Calyptra with abundant to scarce papillose hairs, vaginula (0.25–)0.4–0.8 mm long, naked or with sparse papillose hairs, spores commonly 8–13 µm ………………………………………………………………***O. consimile***

4'. Calyptra naked, exceptionally with very few inconspicuous and smooth hairs, vaginula very long (0.6–)0.8–1.1(–1.3) mm, naked, spores commonly 12–16 µm ………………………………………………………………***O. confusum***

Species description

Orthotrichum consimile Mitt., J. Linn. Soc. Bot. 8: 24. 1864. Lectotype: Canada, British Columbia, Vancouver Island, *Lyall* (NY!).

Figs. IV.4.2(A–G) and IV.4.3(A–H)

Plants 0.3–0.8(–1.0) cm tall in more or less compact tufts, dark green to green-yellowish. *Rhizoids* reddish-brown, smooth, at lower parts and distinctly ascending along stem, often densely so and even reaching the vaginula. *Stems* branched, subpentagonal in section with 1–3 layered sclerodermis of thick-walled cells. *Axillary hairs* (50–)75–175(–200) µm, filiform (2–)3–5 cells long, the 1–2 basalmost brown. *Leaves* 1.7–2.7 x 0.35–0.75 mm, very variable in shape, from lanceolate or linear-lanceolate (often with widened base) to long triangular and sometimes slightly curved, appressed and slightly curved to flexuose when dry, erect-patent when moist; leaf apex remarkably variable, from plane and mucronate-acuminate to folded, unguiculate or long-channeled sometimes also papillose-crenulate, occasionally with a few hyaline elongated or isodiametric cells; leaf margins entire, recurved from base (or lower third) almost to apex, entire or somewhat undulate; leaf lamina unistratose, keeled, especially near apex; costa 52–66(–72) µm wide

Fig. IV.4.2. *Orthotrichum consimile*. A, B: habit; C: leaves; D: capsule before spore release showing operculum; E: young calyptra with long and stout hairs; F: vaginula with sparse, stout hairs. A, F *Norris 103812* (UC); B, C *Dillingham* 2006; E Toren 7212 (CAS).

at base, 33–45 µm at mid-leaf, with 2 rows of ventral cells, ending below leaf apex. Basal leaf cells 25–50(–84) x (7–)9–15 µm, rectangular to elongate, hyaline; marginal basal cells 9–15(–18) x 6–10(–15) µm, rectangular to subquadrate; mid-leaf cells (6–)9–13(–17) x 8–13 µm, isodiametric or oval, rarely oblate, with (1)2(3) usually well developed papillae, very rarely smooth. *Gemmae* scarce on leaves, claviform or fusiform,70–140 x 16–33 µm, 3–8 cells long; rhizoidal gemmae sometimes branched and abundant, vermiform (60–)120–350 x 16–20 µm.

Cladautoicous; perigonia terminal on male branches, perigonial leaves ovate to ovate-lanceolate with rhomboidal cells; perichaetia terminal on female branches, perichaetial leaves not differentiated. *Vaginula* (0.25–)0.4–0.8 mm long, cylindrical to frustum-shaped, naked or with scarce stout, thick-walled, papillose hairs, 160–600 µm long. *Calyptra* conic when young, oblong-conic when mature, yellowish with dark beak, densely or rarely sparsely hairy, with hairs 0.3–1.1 mm long, multiseriate, comprised of short, thick-walled and distinctly papillose cells. *Seta* (0.8–)1.2–2.0(–4.0) mm, twisted counterclockwise. *Capsule* short to long exserted, (0.8–)1.2–1.6 mm in length, oblong to ovoid when immature and full of spores, at maturity urceolate to cylindrical and variably constricted below mouth, strongly ribbed, when dry, pyriform to inflated when moist; capsule neck defluent. *Lid* subconic, with thick, sometimes asymmetric rostrum and orange basal ring. Exothecial cells 18–36(–44) x (7–)11–19 µm, hyaline, elongated;

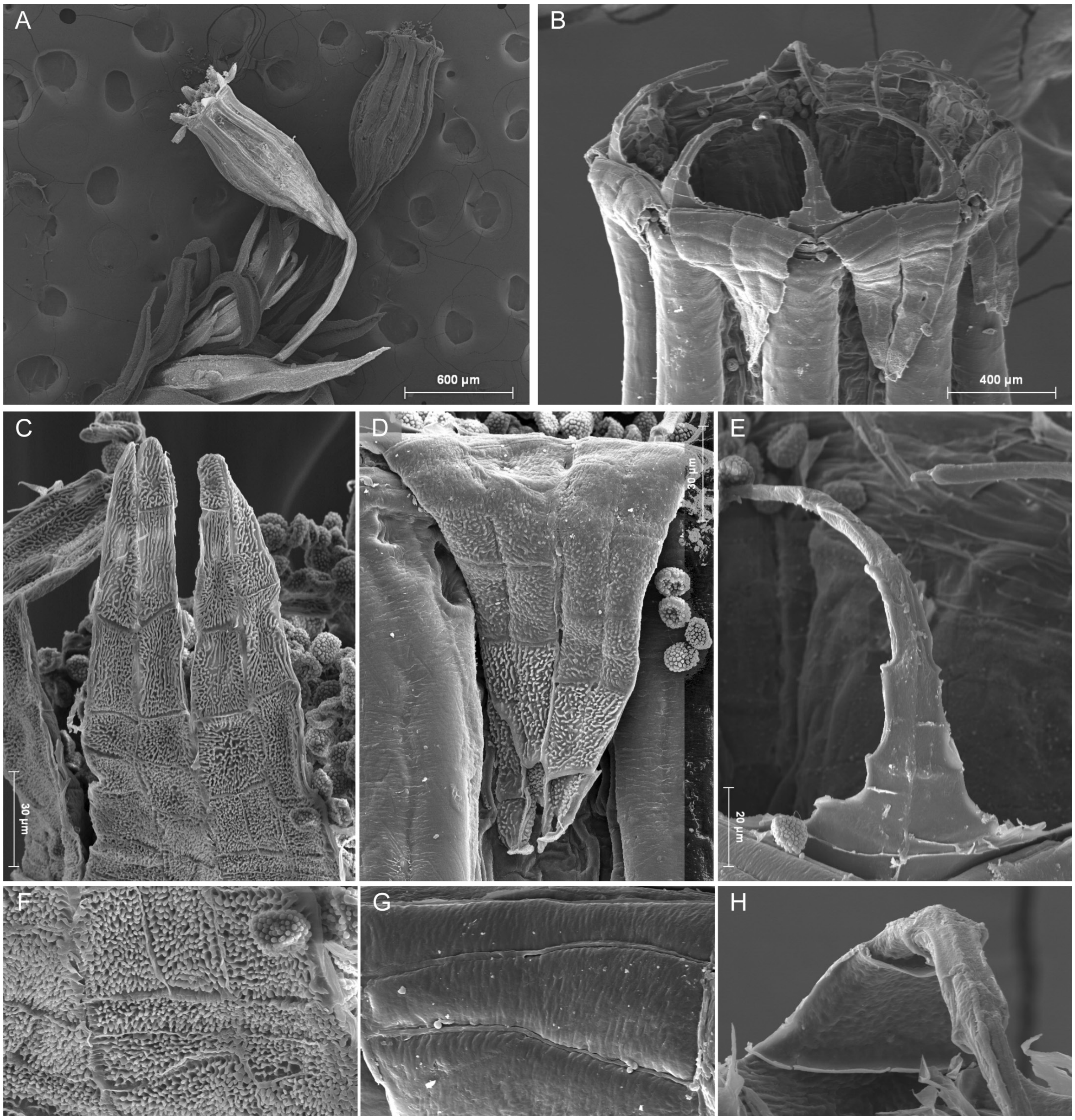

FIG. IV.4.3. *Orthotrichum consimile*. A: capsule; B: detail of the peristome; C: ornamentation of the Outer Peristome Layer (OPL) of an exostome pair of teeth; D: ornamentation of the Principal Peristome Layer (PPL) of an exostome pair of teeth; E: endostome segment smooth PPL; F: detail of the exostome OPL base bearing a papillose reticulum; G: detail of the basal exostome PPL very weakly ornamented; H: endostome segment showing the base of the Inner Peristome Layer (IPL). A, D *Dillingham* 2006; B, E, G, H: *Toren 7212* (CAS).; C, F: *Norris 103812* (UC).

8 exothecial bands differentiated along most of urn, (3–)4–5 cells wide, band cells rectangular (13–)18–35(–54) x 11–21(–28) µm, with thick and coloured walls.*Stomata* cryptopore partially to completely covered by surrounding cells, located in middle urn, sometimes also in lower half, but not reaching neck. *Peristome* double. *Exostome* of 8 pairs of teeth, sometimes splitting when old, recurved when dry, whitish, triangular, (120–)145–200(–220) µm long; external side of teeth (Outer Peristome Layer, OPL) with smooth or variably papillose basal reticule, densely papillose towards apex, often forming vermicular lines aligned longitudinal or radially; internal side of teeth (Primary Peristome Layer, PPL) smooth or very lightly striated at base, rugulose to striate towards apex. *Endostome* of 8 segments, 105–160(–190) µm, linear with wide base, transversal walls

Fig. IV.4.4. *Orthotrichum columbicum*. A: habit; B: capsule at the beginning of the sporosis, full of spores; C: empty capsule; D: capsule before spore release, showing the operculum with the distinct dark red basal ring; E: completely naked calyptrae of young sporophytes; F: variation in leaf shape. A, B, C, F: *Norris 109386* (UC); D: *Garilleti & Lara 7.VII.2011* (Herb. At Univ. Atónoma de Madrid); E: *Lara 5.XII.2001* (Herb. At Univ. Atónoma de Madrid).

sometimes thickened; external side of segments (PPL) smooth or slightly rugulose, intermnal side (Inner Peristome Layer, IPL) smooth or with very light transversal striae towards base; connective membrane continuous and high, ornamented like segment bases. *Spores* 8–13(–17)µm, subspherical, coarsely papillose.

Orthotrichum columbicum Mitt., J. Linn. Soc. Bot. 8: 24. 1864. Type: Canada, British Columbia, Vancouver Island, *Lyall*

> *Orthotrichum winteri* Schimp., Musci Eur. Nov. Bryol. Eur. Suppl. 1 (3–4): 1. 1866. Isotype: Germany, Tholey *Winter* (B!)
>
> *Orthotrichum ulotaeforme* Renauld & Cardot, Bot. Gaz. 15: 42. 1890. Type: Oregon, Coast Mts., *Henderson* (FH!)
>
> *Orthotrichum pulchellum* var. *columbicum* (Mitt.) Grout, Moss Fl. N. Am. 2: 130. 1935.

Orthotrichum pulchellum var. *winteri* (Schimp.) Braithw., Brit. Moss Fl. 2: 88. 1889

Orthotrichum pulchellum var. *longipes* Sull. *ex* Lesq. & James, Man. N. Am. Moss. 175. 1884. Type: Oregon, *Hall*, 1871 (FH! ut *O. columbicum* Mitt.)

Orthotrichum pulchellum var. *productipes* Renauld & Cardot, Bot. Gaz. 15: 43. 1890. Type: Oregon, Portland, *Henderson* (FH!)

Orthotrichum pulchellum ssp. *ulotaeforme* (Renauld & Cardot) Kindb., Eur. N. Am. Bryin. 2: 309. 1897.

Orthotrichum glabrum Venturi *in* Röll, Hedwigia 32: 285. 1893. *Nom. nud*.

Ulota glabra Renauld & Cardot., Bot. Gaz. 15: 42. 1890. *Nom. nud. in synon*.

Figs. IV.4.4(A–F) and IV.4.5(A–G)

Plants 0.4–1.2(–2.2) cm tall in somewhat loose tufts, light green to green-yellowish. *Rhizoids* brown-reddish, smooth or lightly rough, densely covering the lower parts of stems, sometimes variably ascending along them. *Stems* branched in the lower parts, subpentagonal in section, with sclerodermis of 2–3 thick-walled cell layers. *Axillary hairs* 80–130 µm, filiform, 4–6 cells long, the 1–2 basalmost brown. *Leaves* (1.2–)1.7–3.0(–3.5) x 0.25–0.5(–0.7) mm, long triangular to linear-lanceolate or ovate-lanceolate, erect and more or less flexuose to crisped when dry, erect-patent when moist; leaf apex acuminate, sometimes subulate, ending in a blunt point, often with incurved margins forming a variable fold or channel up to 260 µm long; leaf margins entire, recurved in almost their total length; leaf lamina unistratose, keeled in section; costa 35–60(–75) µm wide at base, 35–50 µm at mid-lamina, with 2 rows of ventral cells, ending below leaf apex. Basal leaf cells (15–)25–40(–50) x (7–)10–12 µm, hyaline, rectangular, thick or thin walled; marginal basal cells 10–25 x 7–12(–15) µm, quadrate to rectangular; mid-leaf cells 7–12(–20) x 7–10(–12) µm, isodiametric to lightly oblong, smooth or with 2–3(–4) low papillae. *Gemmae* not observed.

Cladautoicous; perigonia terminal on male branches, perigonial leaves ovate to ovate-lanceolate with rhomboidal cells; perichaetia terminal on female branches, perichaetial leaves not differentiated. *Vaginula* (0.4–)0.5–0.8(–1.2) mm long, cylindrical or slightly fusiform, naked. *Calyptra* narrowly conic to fusiform when young, distinctly long conical when mature, yellowish with dark beak, naked. *Seta* (2.0–)2.5–5.5 mm, twisted counterclockwise. *Capsule* long exserted, (1.25–)1.5–1.7(–2.25) mm in length, cylindrical to fusiform and usually somewhat constricted below mouth, when full of spores, gradually constricted from mouth to lower half (third) of the urn and strongly ribbed as

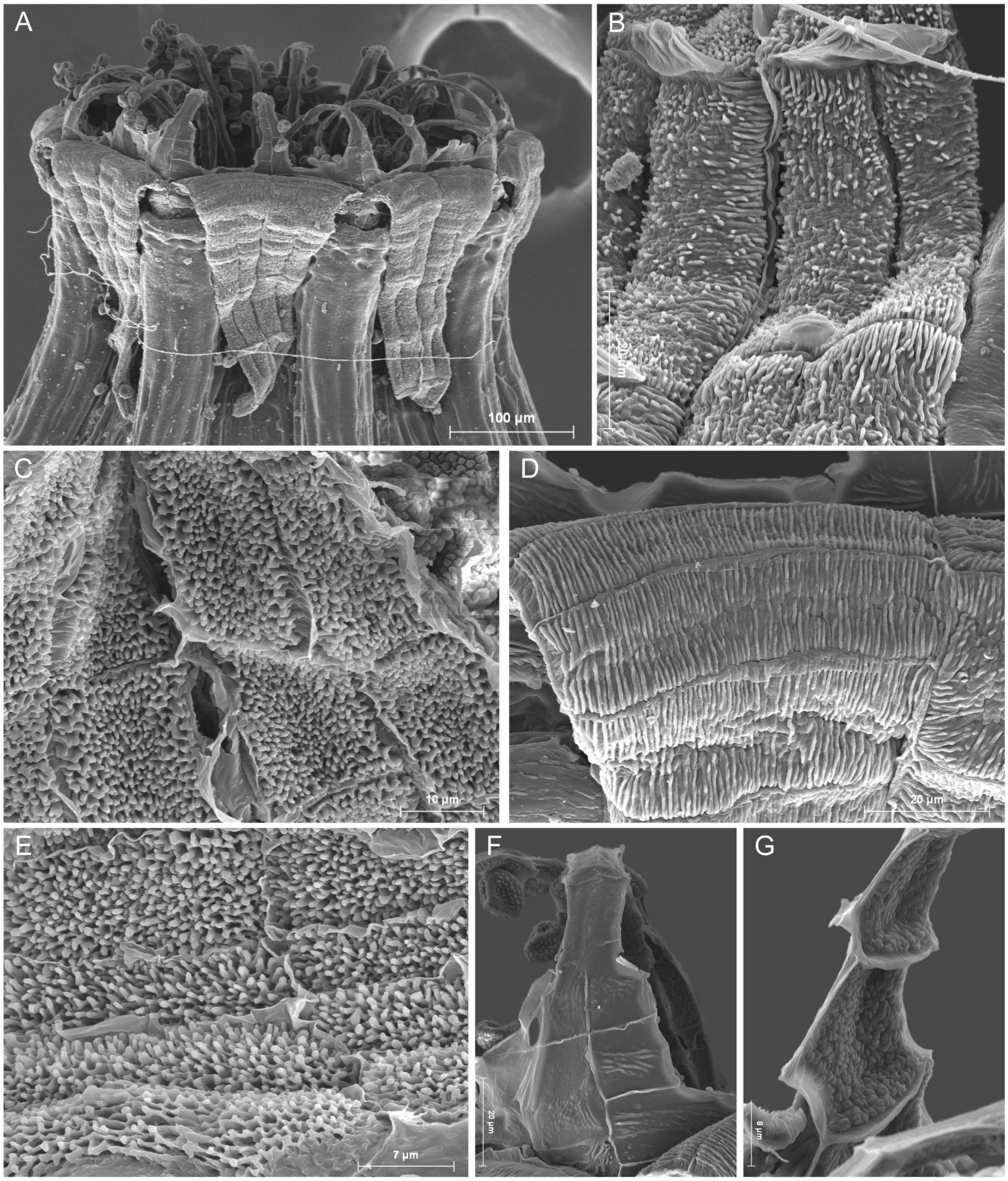

Fig. IV.4.5. *Orthotrichum columbicum*. A: detail of the capsule mouth; B: ornamentation on the apical exostome PPL; C: densely papillose ornamentation on the apical exostome OPL; D: distinct longitudinal lines on the basal exostome PPL; E: basal exostome OPL ornamentation; F: endostome segment showing sparse lines on the PPL; G: inner surface (IPL) of an endostome segment showing low papillae. A, B, D, F: *Norris 109386* (UC); C, E: *Lara & Garilleti 20.VII.2000* (Herb. At Univ. Atónoma de Madrid); F: *Estébanez 25.VI.2010* (Herb. At Univ. Atónoma de Madrid).

the urn empties, ovoid when moist; capsule neck defluent. *Lid* subconic to almost plane, same colour as capsule except for a distinct wide, bright red to crimson basal ring, with thick rostrum. Exothecial cells (14–)20–45(–55) x 12–20(–25) µm, hyaline, elongated; 8 exothecial bands differentiated along most of urn, (3–)4–6 cells wide, with band cells (20–)25–40(–60) x (12–)14–27(–30) µm, rectangular, brownish and thick-walled. *Stomata* cryptopore partially to almost completely covered by surrounding cells, located in middle and lower half of urn, not reaching the neck. *Peristome* double. *Exostome* of

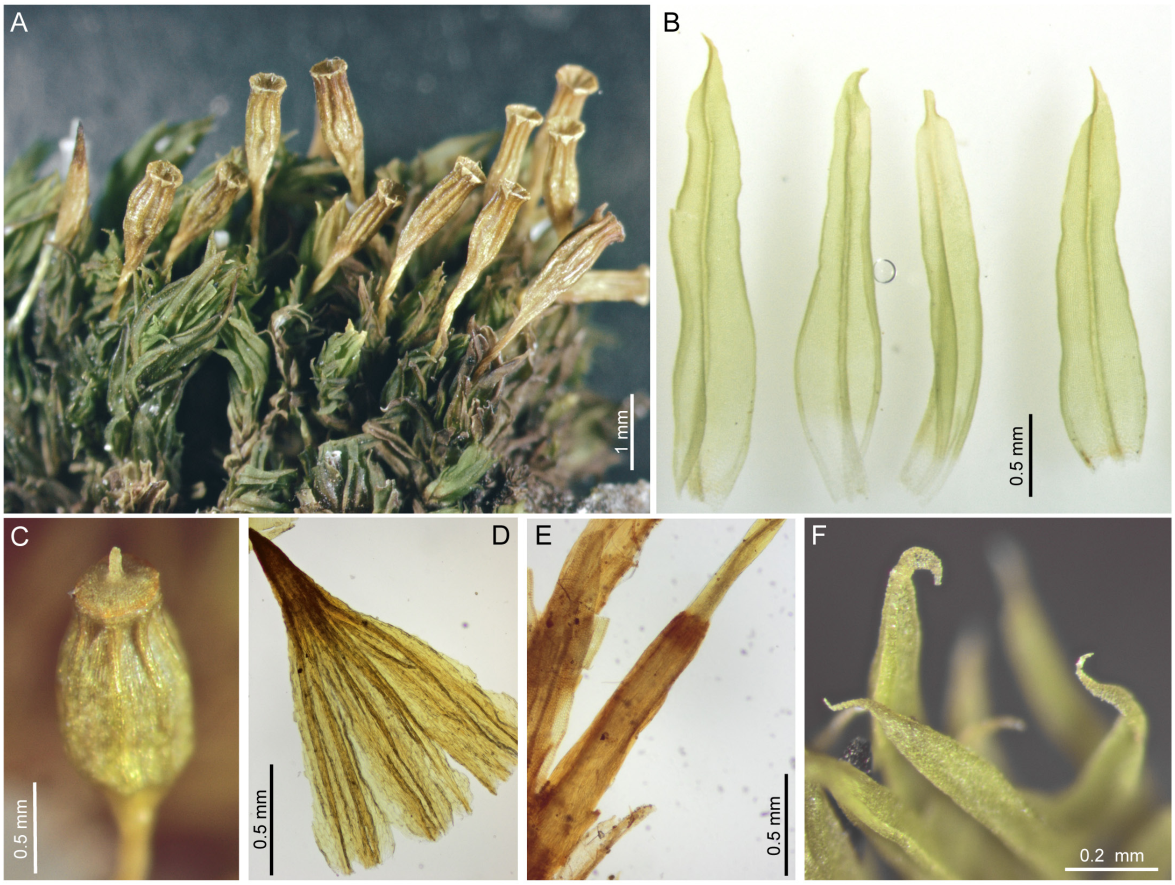

Fig. IV.4.6. *Orthotrichum confusum*. A: habit; B: leaf variation; C: capsule before spore release; D: naked calyptra; E: typical long and completely naked vaginula; F: distinct unguiculate leaf apices. A, B: holotype (UC); C, D, F: *Dillingham 1983 & Toren*; E: *Norris 108392* (UC);

8 pairs of teeth, sometimes splitting when old, reflexed when dry, distinctly whitish or pale yellowish, long triangular, (200–)225–280 µm long, with apical cell walls sometimes incrassate; OPL densely papillose even at basal reticulum, sometimes forming lines in the apex, PPL with well marked longitudinal striae at base, papillose above. *Endostome* of 16 linear, sometimes appendiculate segments, 175–250(–280) µm, sometimes the intermediate can be reduced, most times 50–160 µm), very rarely only 8; PPL slightly striated or smooth; IPLpapillose or smooth; connective membrane continuous and high, ornamentated as segments. *Spores* (12–)14–17(–20) µm, subspherical, papillose.

Orthotrichum confusum R. Medina, F. Lara & Garilleti *sp. nov.*

> *Plantae ad 1.3 cm altae, rhizoideis laevibus ascendentibus secus caules, subinde propagulis munitis. Folia in sicco flexuosa, linearia vel elliptica-lanceolata, marginibus laxe recurvatis, apicibus variabillimis: acuminatis, canaliculatis vel unguiculatis. Vaginula nuda, cylindricave (0.6–)0.8–1.1(–1.3) mm longa. Calyptra persaepe nuda. Capsula exserta, urceolata vel cylindrica, 8-striata, stomatibus cryptoporis in area centrali. Exostoma octo dentium paribus recurvatis, endostoma octo segmentis. Sporae grosse papillatae, 12–16(–20) µm diametro.*

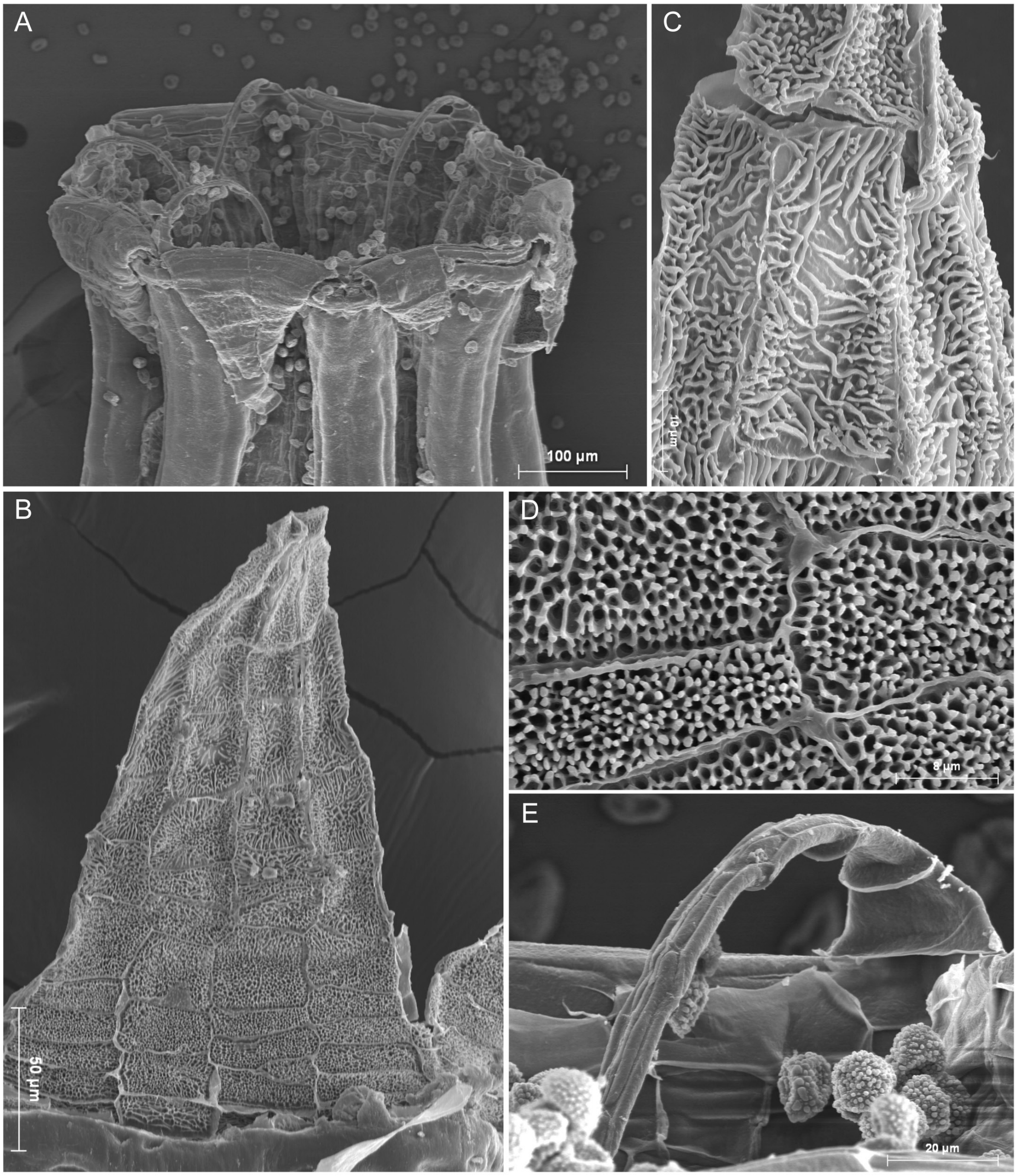

Fig. IV.4.7. *Orthotrichum confusum*. A: mouth of the capsule; B: complete exostome teeth pair showing OPL ornamentation; C: detail of the ornamentation of the exostome apical OPL; D: detail of the ornamentation of the exostome basal OPL; E: smooth endostome segment and connective membrane. A, E: *Norris 108392* (UC); B, C, D: *Dillingham 1983 & Toren*;

HOLOTYPE: U.S.A. Siskiyou Co. 41º56'31"N 122º08'42"W, on branches of *Abies magnifica*, along Pacific Crest Trail east of Cook & Green Pass north of Seiad Valley, Klamath National Forest, elev. 1440-1744 m; 17 September 2004, *D.H. Norris 108391 & N. Hillyard* (UC-1769994). Isotype at Universidad Autónoma de Madrid.

Figs. IV.4.6(A–F) and IV.4.7(A–E)

Plants 0.3–1.0(–1.3) cm tall, in more or less compact tufts, dark green to green-yellowish. *Rhizoids* reddish-brown, smooth, at lower parts and ascending along the stem even up to the vaginula, sometimes forming a very dense tomentum, occasionally propaguliferous. *Stems* branched, subpentagonal in section, with a 2–3 layered sclerodermis of thick-walled cells. *Axillary hairs* 80–150(–225) µm, filiform or subclaviform, 3–5 cells long, the 1–2 basalmost brown. *Leaves* 1.75–3.25 x 0.25–0.65 mm, very variable in shape: linear-lanceolate, long triangular or elliptic-lanceolate, even slightly asymmetrical or subfalcate, appressed and slightly flexuose when dry, erect-patent when moist; leaf apex very variable, frequently with a filiform to unguiculate acumen, also long channeled or twisted; leaf margins, entire, loosely recurved to revolute in most of their length; leaf lamina unistratose, keeled in section; costa (40–)55–70 µm wide at base, 32–50 µm at mid-leaf, reniform in section, with two rows of ventral cells, ending below apex; lamina unistratose. Basal leaf cells 34–77(–100) x (8–)13–19 µm, hyaline, rectangular to elongate, thick or thin walled, sometimes nodulose; marginal basal cells 9–17(–23) x 11–15 µm rectangular to subquadrate with thickened walls; mid-leaf cells 9–18 x 9–13(–16) µm, isodiametric or somewhat elongate, with 2–3(–4) simple or branched papillae. *Gemmae* scarce on leaves, fusiform or baciliform, brownish, (50–)65–125 x 21–28 µm, 3–6 cells long; rhizoidal gemmae vermiform, sometimes branched, 115–305 x 22–28 µm long.

Cladautoicous; perigonia terminal on male branches, perigonial leaves ovate-acuminate with rhomboidal cells; perichaetia terminal on female branches, perichaetial leaves not differentiated. *Vaginula* (0.6–)0.8–1.1(–1.3) mm, long doliiform or cylindrical, naked and often with persistent archegonia. *Calyptra* conic when young, turning oblong-conic to fusiform when mature, with a dark beak, naked or very rarely with isolated inconspicuous short and smooth hairs. *Seta* 1.1–1.8(–2.3) mm, twisted counterclockwise, bent down when very long. *Capsule* short to long exserted, (0.6–)1.5–2.0 mm in length, ovoid when immature and full of spores, urceolate to cylindrical and variably constricted below mouth during and after sporosis, strongly ribbed, pyriform or ovoid when moist; capsule neck defluent. Lid almost plane to subconic, rostrate, yellowish, with orange basal ring. Exothecial cells (18–)25–47(–60) x 12–25(–32) µm, hyaline, elongated; 8 exothecial bands differentiated along most of urn, (3–)4(–5) cells wide, with band cells (15–)21–53 x (13–)20–35 µm, rectangular, brown and thick-walled. *Stomata* cryptopore, partially to completely covered by surrounding cells, located in middle of urn, rarely also in lower half but absent on neck. *Peristome* double. *Exostome* of 8 pairs of teeth, rarely splitting when old, whitish or pale brown, triangular, 225–270 µm long; OPL with a basal reticule, more or less papillose, and dense papillae towards apex, often forming vermicular lines disposed longitudinally, transversally or radially, apical cell walls sometimes thickened; PPL smooth or slightly rugulose. *Endostome* of 8 linear segments, (140–)200–230 µm; PPL smooth; IPL smooth or slightly rugulose; connective membrane continuous but low. *Spores* 12–16(–20) µm, subspherical, coarsely papillose.

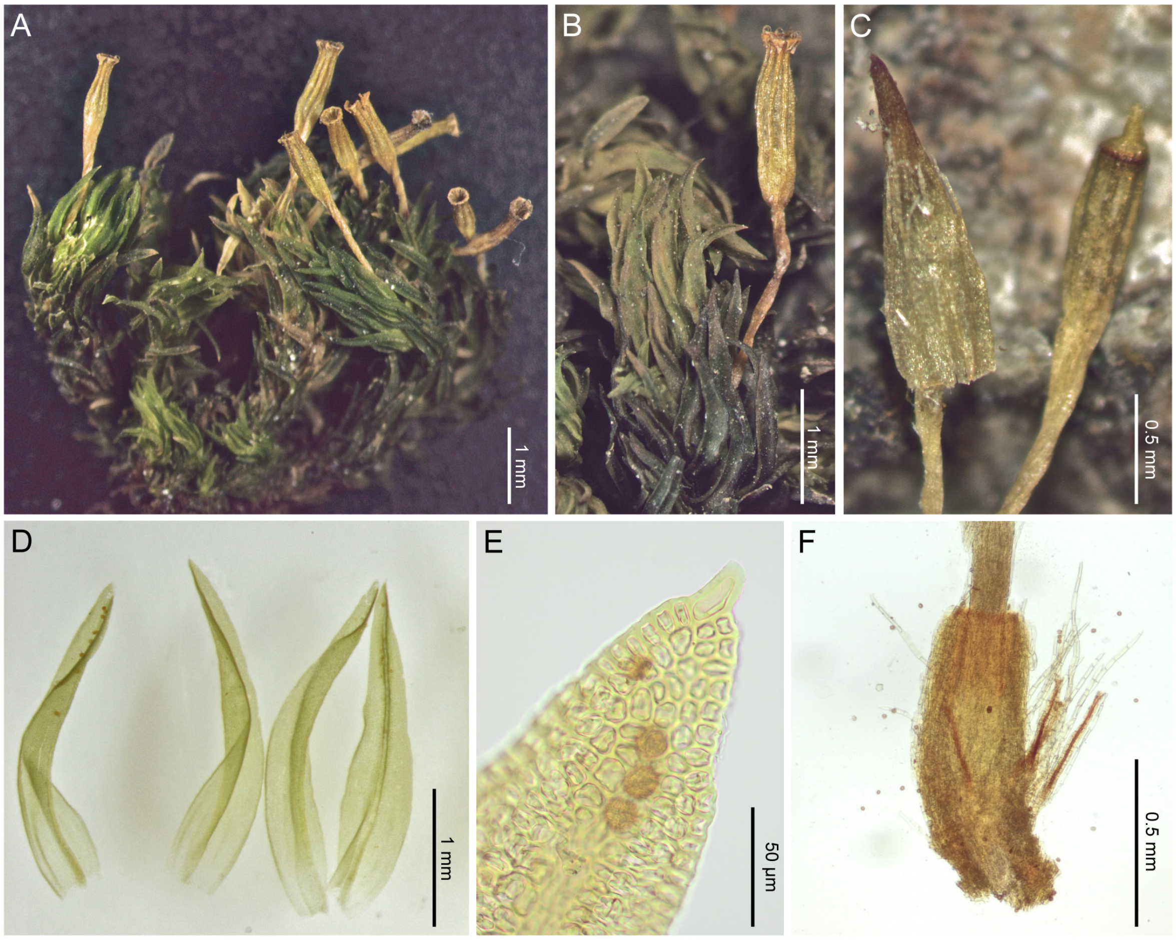

Fig. IV.4.8. *Orthotrichum persimile*. A: habit; B: gametophore and capsule; C: calyptra with sparse hairs and capsule before sporosis bearing the operculum; D: leaf variation; E: detail of leaf apex with the distinct apical hyaline cell; F: hairy vaginula. A: *Lara & Garilleti 28.IX.2008* (Herb. At Univ. Atónoma de Madrid); B: *Kellman 791*; C-F: holotype (UC).

Etymology:

The specific epithet remarks the confusion that this species caused when it was discovered unexpectedly by the phylogenetic analyses.

Orthotrichum persimile* F. Lara, R. Medina & Garilleti *sp. nov.

> *Plantae usque ad 1.0 cm altae, rhizoideis scabris. Folia in sicco flexuosa, elliptica-lanceolata, marginibus apicibusque planis, in pauculas cellulas hyalinas terminantia. Vaginula dense pilosa, sed calyptra dispersim pilifera. Capsula cylindrica, exserta, 8-striata, stomatibus cryptoporis in dimidio inferiore. Peristoma duplex. Exostoma octo dentium paribus recurvatis. Endostoma octo segmentis affixis ad basem per altam membranam.*

HOLOTYPE: U.S.A. California. Contra Costa Co. Wildcat Canyon 37º56’01”N 122º17’44”W; trunk of *Aesculus californica*, 20 September 2008, *F. Lara, R. Garilleti & D. H. Norris* (CAS). Isotype at Universidad Autónoma de Madrid.

Figs. IV.4.8 (A–F) and IV.4.9 (A–G)

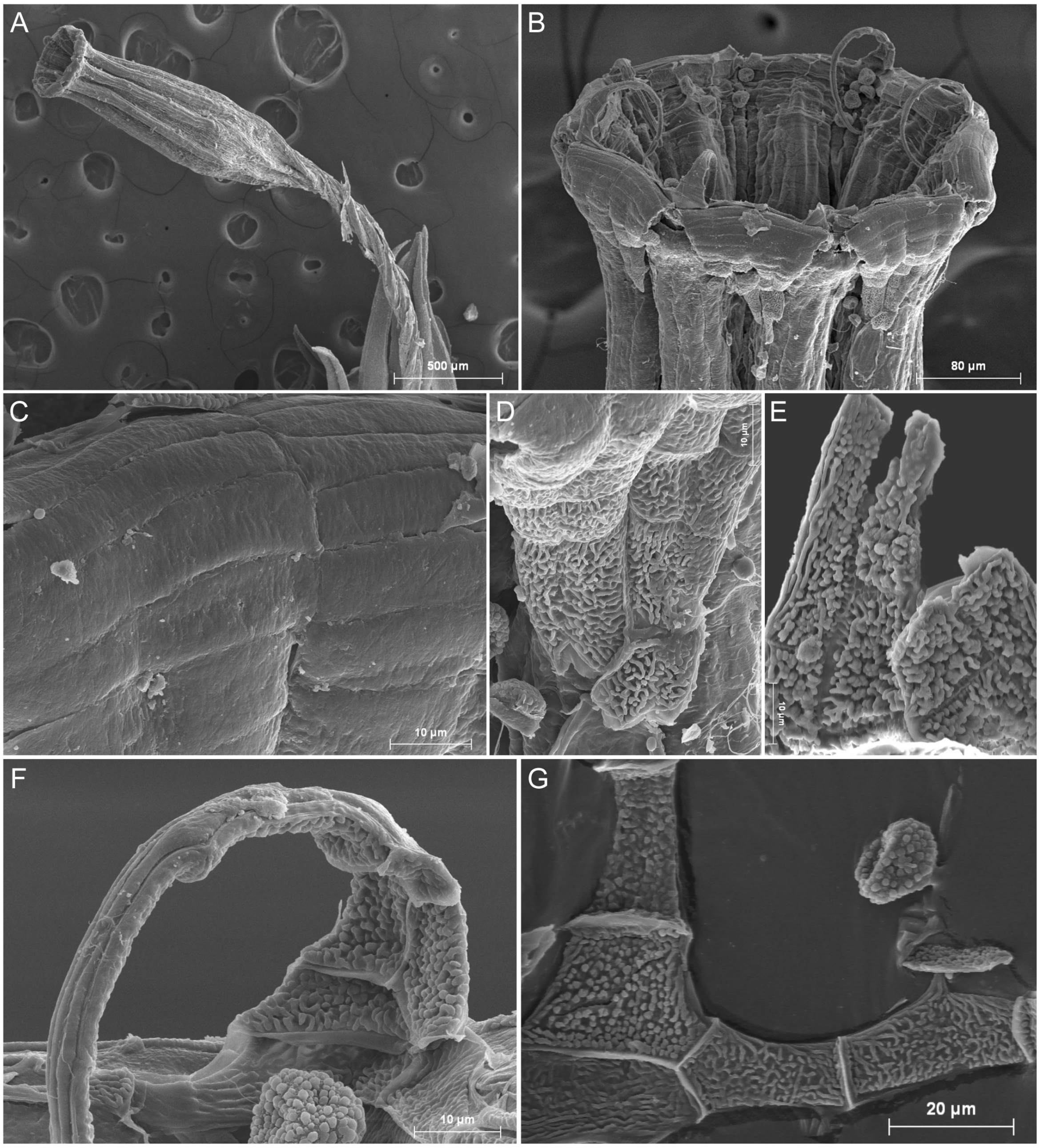

Fig. IV.4.9. *Orthotrichum persimile*. A: view of a sporophyte; B: detail of the capsule mouth; C: detail of the smooth basal exostome PPL; D: apical exostome PPL showing vermicular lines; E: detail of the ornamentation of the apical exostome OPL consisting on coarse papillae and lines; F: endostome segment showing a smooth PPL and a IPL ornamented with coarse papillae; G: detail of the IPL ornamentation of the base of a endostome segment and the connective membrane consisting on papillae and transversal striae. A-F: holotype (UC); G: *Lara & Garilleti 28.IX.2008* (Herb. At Univ. Autónoma de Madrid);

Plants 0.4–1.0 cm tall in loose tufts, yellowish to olive green. *Rhizoids* reddish brown, rough, scarce and restricted to lower parts of stems. *Stems* branched, subpentagonal in section, with sclerodermis of 2–3(–4) thick-walled cell layers. *Axillary hairs* 90–175(–225) µm, 3–6 cells long, the basalmost 1–2 brown. *Leaves* 1.2–2.2(–2.7) x 0.3–0.5(–0.65) mm, elliptic-lanceolate to linear-lanceolate, appressed and slightly flexuose when dry, erect-patent when moist; leaf apex acute to slightly obtuse, usually briefly apiculate in young leaves with a large terminal hyaline cells; leaf margins plane, sometimes most linear-shaped leaves with one (rarely both) recurved in lower half (third); leaf lamina unistratose, somewhat keeled in section; costa 30–60(–80) µm wide at base, 35–50 µm at mid-leaf, with 2 rows of ventral cells, ending below apex. Basal leaf cells (12–)18–72(–90) x 7–10(–15) µm, hyaline, more or less rectangular to elongate, walls thin, seldom somewhat thickened; marginal basal cells 12–25(–37.5) x (5–)7.5–10 µm, hyaline or chlorophyllose, rectangular to quadrate; middle and leaf cells (7–)10–18(–22) x (6–)7.5–12 µm, isodiametric to elongate-elliptical, with 2–4 papillae, especially in upper leaf half, rarely smooth. *Gemmae* occasionally present on leaves, fusiform to claviform, brownish, 40–72(–120) x 20–30 µm, 3–6(–8) cells long.

Cladautoicous; perigonia lateral or terminal on male branches, perigonial leaves ovoid to ovate–lanceolate with rhomboidal cells; perichaetia terminal on female branches, perichaetial leaves not differentiated. *Vaginula* 0.5–0.75mm long, cylindrical, with numerous 1(2)-seriate hyaline hairs, thin to somewhat thick-walled, smooth or faintly papillose, 250–600 µm long. *Calyptra* fusiform when young, oblong-conic when mature, green-yellowish, slightly ribbed, with scattered hairs similar to those of the vaginula. *Seta* 1.2–1.7 mm long, yellowish, twisted counterclockwise. *Lid* subconic, with a stout rostrum and wide darker basal ring. *Capsule* more or less shortly exserted, 1.45–1.75 mm in length, cylindrical when full of spores, cylindrical, strongly ribbed and variably constricted below mouth when empty and dry, cylindrical to pyriform when empty and moist; capsule neck defluent. Exothecial cells (20–)35–50(–80) x 9–25 µm, hyaline, elongated; 8 exothecial bands differentiated along most of urn, 3–4(–5) cells wide, band cells (12–)15–30(–46) x (8–)12–20(–27) µm, darker, thick-walled. *Stomata* cryptopore, almost to completely covered by surrounding cells, restricted to the lower half of the urn and neck. *Peristome* double. *Exostome* of 8 pairs of teeth, non-splitting when old, recurved when dry, whitish to pale orange, triangular, 170–240 µm long; OPL with a reticulum densely covered by low papillae, towards apex also papillose, sometimes with vermicular lines; PPL smooth at base, with faint lines and papillae above. *Endostome* of 8 segments, 150–170 µm long, stout and wide at base; PPL smooth; IPL slightly to clearly reticulate-papillose; connecting membrane, completely developed and distinctly high. *Spores* 15–17(–20) µm, subspherical, densely papillose.

Etymology

The specific name, meaning “extremely similar”, stresses the difficulty to distinguish the species from its allies mentioned in this work.

New synonym

***Orthotrichum pulchellum* Brunt.**

Orthotrichum hendersonii Renauld & Cardot, Bot. Gaz. 15: 42. 7A. 1890. Type: Oregon, Coast Mts., *Henderson* (Lectotype – FH-herb.Cardot!) ***syn. nov***.

Ulota hendersonii Renauld & Cardot., Bot. Gaz. 15: 42. 1890. *Nom. nud. in synon*.

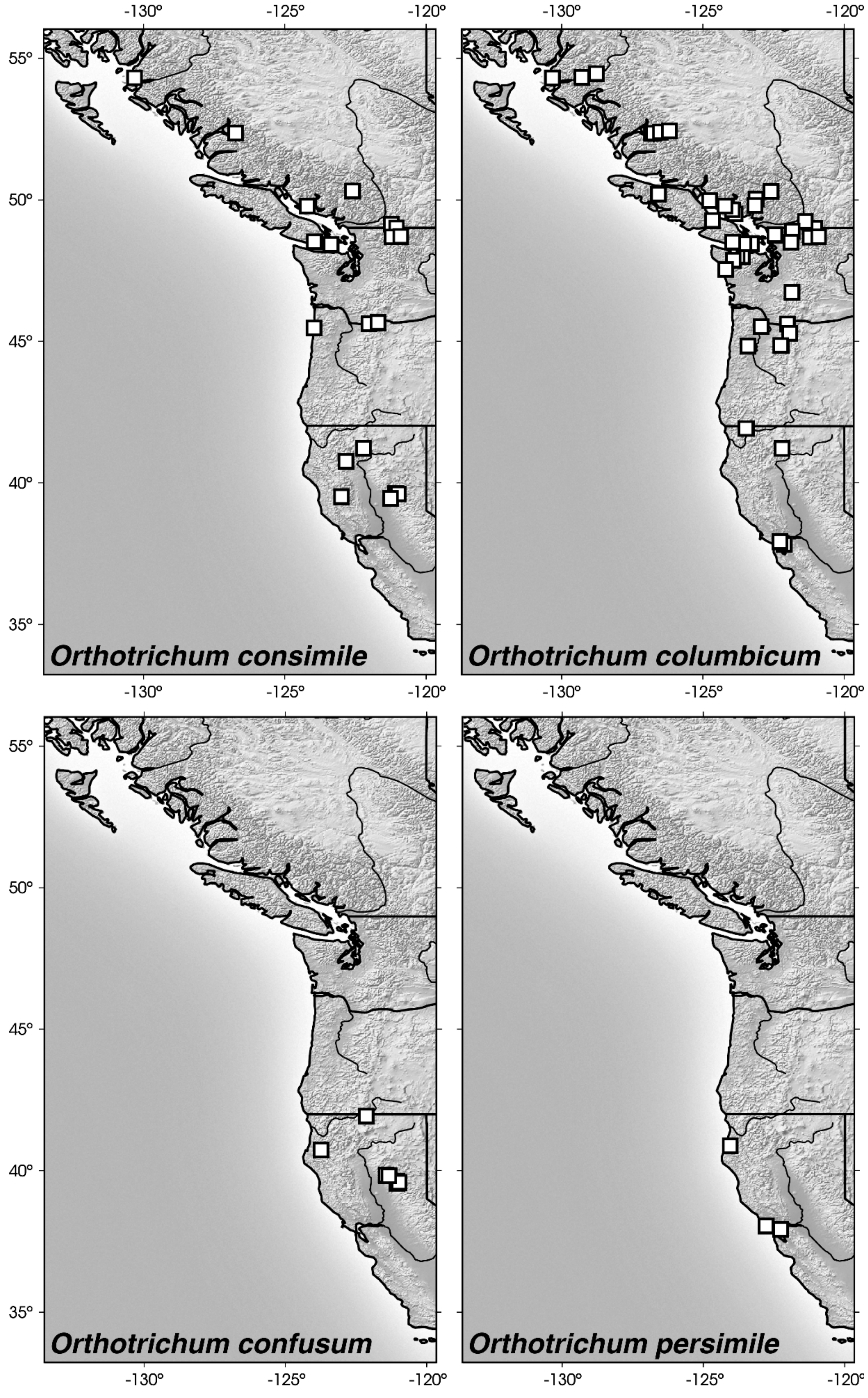

Fig. IV.4.10. Known distribution of the studied species: *Orthotrichum consimile*, *O. columbicum* (only the American distribution is shown), *O. confusum* and *O. persimile*.

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Appendix

Selection of specimens including voucher information (according to the guidelines, GenBank accession numbers will be provided after the manuscript is accepted). Vouchers without herbarium acronyms are kept at authors' herbarium Universidad Autónoma de Madrid. Specimens shown in Fig. IV.4.1 are preceded by a bracketed number.

Orthotrichum consimile. **CANADA. British Columbia:** Victoria, Beacon Hill Park 3.XII.2001 *F. Lara*; Skeena-Queen Charlotte Regional District, 54º18’32’’ N, 130º19’50’’ W, alt. 40 m 9.VIII.2011 *R. Garilleti & F. Lara*; Central Coast Regional District 52º22’18’’ N, 126º45’22’’ W, alt. 20 m 6.VIII.2011 *R. Garilleti & F. Lara*; Squamish-Lillooet Regional District, 50º18’24’’ N, 122º36’16’’ W, alt. 216 m 5.VIII.2011 *R. Garilleti & F. Lara*; Fraser Valley Regional District 49º07’57’’ N, 121º13’58’’ W, alt. 580 m 20.VII. 2011 *F. Lara, R. Garilleti & B. Albertos*; Vancouver Island, Capital Regional District, 48º30’18’’ N, 123º57’27’’ W, alt. 400 m 13.VIII.2011 *R. Garilleti & F. Lara*; Vancouver Island, Capital Regional District 48º25’05.7’’ N, 123º21’56.6’’ W, alt. 50 m 13.VIII.2011 *R. Garilleti & F. Lara*; Powell River Regional District 49º46’56’’ N, 124º13’03’’ W, alt. 6 m 19.VII. 2011 *F. Lara, R. Garilleti & B. Albertos*; **U.S.A. California:** Lake Co. **[2]** Mendocino N.F. 28.VI.1998 *D. Toren 7212* (CAS-958593); Shasta Co. **[3]** 6 miles S of Dunsmuir 10.V.2002 *D. H. Norris 103812 pro parte* (UC-1760062); Plumas Co.**[1]** Plumas N.F. 2.VI.2005 *C. Dillingham 2006*; Sierra Co. Plumas N.F. 15.IV.2005 *C. Dillingham 1884 pro parte*; Trinity Co. Trinity N.F. 19.XII.1984 *D. H. Norris 71953* (UC-1629890); Yuba Co. Plumas N.F. 19.XII.2003 *C. Dillingham 1204*; **Oregon:** Hood River Co. **[4]** Viento S.P. 27.III.2008 *D. Toren*; Tillamook Co., 45º27’40’’ N, 123º58’13’’ W F. 27.VII.2011 *F. Lara, R. Garilleti & B. Albertos*; **Washington:** Whatcom Co. Ross Lake 48º59’53’’ N, 121º03’45’’ W, alt. 536 m. 20.VII.2011 *F. Lara, R. Garilleti & B. Albertos*; North Cascades National Park 48º41’56’’ N, 121º12’32’’ W, alt. 362 m. 22.VII.2011 *F. Lara, R. Garilleti & B. Albertos*; Okanogan National Forest, Canyon Creek Trail, 48º42’24’’ N, 120º55’03’’ W, alt. 575 m. 22.VII.2011 *F. Lara, R. Garilleti & B. Albertos*; Skamania Co. Columbia River 45º37’14’’ N, 122º01’25’’ W, alt. 20 m. 26.VII.2011 *F. Lara, R. Garilleti & B. Albertos*.

Orthotrichum columbicum. **CANADA. British Columbia:** Goldstream 21.VII.1950 *Flowers* (COLO-339783); **[4]** Victoria, Campus of the Univ. Of V. 5.XII.2001 *F. Lara*; McMillan Provincial Park 7.XII.2001 *F. Lara*; Regional District of Kitimat-Stikine, Skeena River 54º28’11’’ N, 128º46’23’’ W, alt. 120 m. 9.VIII-2011 *R. Garilleti & F. Lara*; Exchamsiks River Provincial Park, 54º20’16’’ N, 129º17’43’’ W, alt. 19 m. 9.VIII.2011 *R. Garilleti & F. Lara*; Skeena-Queen Charlotte Regional District, Kaien Island, 54º18’32’’ N, 130º19’50’’ W, alt. 40 m. 9.VIII.2011 *R. Garilleti & F. Lara*; Central Coast Regional District, Bella Coola town, 52º22’18’’ N, 126º45’22’’ W, alt. 20 m 6.VIII.2011 *R. Garilleti & F. Lara*; 52º22’13’’ N, 126º48’52’’ W, alt. 5 m. 7.VIII.2011 *R. Garilleti & F. Lara*; Hagensborg 52º23’30’’ N, 126º30’47’’ W, alt. 85 m. 7.VIII.2011 *R. Garilleti & F. Lara*; Tweedsmuir Provincial Park 52º26’07’’ N, 126º11’25’’ W, alt. 180 m. 7.VIII.2011 *R. Garilleti & F. Lara*; Regional District of Mount Waddington 50º12’45’’ N, 126º35’41’’ W, alt. 200 m. 12.VIII.2011 *R. Garilleti & F. Lara*; Capital Regional District 48º30’18’’ N, 123º57’27’’ W, alt. 400 m. 13.VIII.2011 *R. Garilleti & F. Lara*; Powell River Regional District, Malaspina Peninsula 49º46’56’’ N, 124º13’03’’ W, alt. 6 m. 19.VII.2011 *F. Lara, R. Garilleti & B. Albertos*; Lund 49º59’15’’ N, 124º46’26’’ W, alt. 48 m. 19.VII.2011 *F. Lara, R. Garilleti & B. Albertos*; Sunshine Coast Regional District, Sechelt Peninsula 49º38’47’’ N, 123º58’07’’ W, alt. 88 m. 18.VII.2011 *F. Lara, R. Garilleti & B. Albertos*; Little Knives 49º30’20’’ N, 123º52’29’’ W, alt. 70 m. 18VII.2011 *F. Lara, R. Garilleti & B. Albertos*; Squamish-Lillooet Regional District, Squamish Valley 49º48’03’’ N, 123º09'30'' W, alt. 50 m. 5.VIII.2011 *R. Garilleti & F. Lara*; Brandywine Falls Provincial Park, 50º02'16'' N, 123º07'14'' W, alt. 460 m. 5.VIII.2011 *R. Garilleti & F. Lara*; ca. 10 km E of Pemberton 50º18'24'' N, 122º36'16'' W, alt. 216 m. 5.VIII.2011 *R. Garilleti & F. Lara*; Fraser Valley Regional District, Skagit Valley Provincial Park 49º13'50'' N, 121º23'01'' W, alt. 454 m. 20.VII.2011 F. *Lara, R. Garilleti & B. Albertos*; **U.S.A. California: [1]***D. Toren 7147* (CAS-958588); Contra Costa Co. Wildcat Canyon 37º56'01"N 122º17'44"W 20.IX.2008 *F. Lara, R. Garilleti & D. H. Norris;* **[2]** Contra Costa Co. 37º49.1'N 122º08.5'W 1.III.2007 *D. H. Norris 109730* (UC-1917669); **[3]** Shasta Co. 6 miles S of Dunsmuir 10.V.2002 *D. H. Norris 103812 pro parte* (UC-1760062); Syskiyou Co. 41º56'N 123º29'W 31.X.1989 *D. H. Norris 75764*; **Oregon:** *Hall* 1871 (TR-Venturi); Clackamas Co., Mt Hood National Forest 45º17'28'' N, 121º56'24'' W, alt. 495 m. 26.VII.2011 *F. Lara, R. Garilleti & B. Albertos*; **[5]** Marion Co. Willamette N.F. 44º51.4'N 122º14.8'W 5.IX.2006 *D. H. Norris 109386* (UC); Polk Co. Little Sink N.A. 44º51'N 123º25'W 22.I.1993 *D. H. Norris 80254*; Washington Co., Hillsboro 45º31'53'' N, 122º56'39'' W, alt. 58 m. 28.VII.2011 *F. Lara, R. Garilleti & B. Albertos*; **Washington:** Clallam Co. Olympic National Park 47º59'58'' N, 123º36'47'' W, alt. 293 m. 23.VII.2011 *F. Lara, R. Garilleti & B. Albertos*; Lake Crescent 48º03'30'' N, 123º47'16'' W, alt. 190 m. 23.VII.2011 *F. Lara, R. Garilleti & B. Albertos*; Jefferson Co. Olympic National Park, 47º51'48'' N, 123º56'02'' W, alt. 215 m. 24.VII.2011 *F. Lara, R. Garilleti & B. Albertos*; Lower Queets Valley Road 47º33'06'' N, 124º12'12'' W, alt. 55 m. 24.VII.2011 *F. Lara, R. Garilleti & B. Albertos*; Skagit Co. Rasar State Park 48º30'51'' N, 121º54'21'' W, alt. 50 m. 22.VII.2011 *F. Lara, R. Garilleti & B. Albertos*; Pierce Co., Mt Rainier National Park 46º44'11'' N, 121º51'20'' W, alt. 750 m. 25.VII.2011 *F. Lara, R. Garilleti & B. Albertos*; Skamania Co. Beacon Rock State Park 45º37'14'' N, 122º01'25'' W, alt. 20 m. 26.VII.2011 *F. Lara, R. Garilleti & B. Albertos*; Whatcom Co., Ross Lake 48º59'53'' N, 121º03'45'' W, alt. 536 m. 20.VII.2011 *F. Lara, R. Garilleti & B. Albertos*; Mt Baker Hwy 48º54'23'' N, 121º50'45'' W, alt. 388 m. 21.VII.2011 *F. Lara, R. Garilleti & B. Albertos*; Bellingham 48º46'18'' N, 122º27'45'' W, alt. 50 m. 21.VII.2011 *F. Lara, R. Garilleti & B. Albertos*; North Cascades National Park 48º41'56'' N, 121º12'32'' W, alt. 362 m. 22.VII.2011 *F. Lara, R. Garilleti & B. Albertos*; Okanogan National Forest 48º42'24'' N, 120º55'03'' W, alt. 575 m. 22.VII.2011 *F. Lara, R. Garilleti & B. Albertos*; **SPAIN. Álava:** Luquiano 12.IV.1997 *B. Albertos & R. Garilleti;* Barranco de Igoroen 26.I.1997 *B. Albertos et al.*; **Asturias:** Cerredo 31.III.1996 *B. Albertos et al.*; **[6]** Tejera del Sueve 43º27'06"N 05º15'06"W 25.VI.2010 *B. Estébanez*; **León: [7]** Río Selmo 20.VII.2000 *F. Lara & R. Garilleti*; **La Coruña:** Santa Comba 31.III.1996 *B. Albertos et al.*; **León:** Paradaseca 01.IV.1996 *B. Albertos et al.*; **Lugo:** Devesa de Rogueira UTM 29TPH5419 19.VIII.1998 *B. Albertos, I. Draper & V. Mazimpaka*;

***Orthotrichum confusum*. U.S.A. California:** Butte Co. Lassen N. F. 16.XI.2004 *C. Dillingham 1790*; Humboldt Co. 40º45'N 123º45'W 29.X.1977 *D. H. Norris 50376* (UC); Nevada Co. Greenhorn Creek 13.VII. 1933 *MacFadden* (UC-1802277); Plumas Co. Plumas N.F. 9.VII.2009 *C. Dillingham 2596*; Wildcat Creek 39º49'25"N 121º20'25"W 30.IX.2009 *Toren 9721*; Sierra Co. Plumas N.F. 15.IV.2005 *C. Dillingham 1884 (pro parte)*; Siskiyou Co. **[1]** Klamath National Forest, 41º56'31"N 122º08'42"W 17.IX.2004, *D. H. Norris 108392, 108390* (UC-1769993, 1769995); Yuba Co. **[2]** Plumas N.F. 26.V.2005 *Dillingham 1983 & Toren*.

***Orthotrichum persimile*. [1 and 3 from holotype] U.S.A. California:** Humboldt Co. Azalea Reserve S.P. .XI.1976 *D. H. Norris 48207* (UC-1650645); .Marin Co. Olema 38º02'30"N 122º48'00"W 28.IX.2008 *F. Lara & R. Garilleti*; 11.I.1894 *Howe* (TR-VENTURI); Santa Cruz Co. **[2]** Big Basin Redwoods S.P. 37º05'50"N 122º16'30"W 4.IX.1999 *K. M. Kellman 791*.

***Orthotrichum acuminatum*. SPAIN. Gran Canaria:** Between Roque Nublo and Pico de las Nieves 22.IV.2004 *R. Medina*; ***Orthotrichum affine*. SPAIN. Burgos:** Merindad de Sotoscueva 12.VI.2008 *R. Medina*; ***Orthotrichum alpestre*. U.S.A. Colorado: [1]** Rio Grande Co. 37º33'N 106º42'W 12.VIII.1990 *Vitt Exs. 47* (DUKE); ***Orthotrichum anomalum*. SPAIN. Asturias: [3]** Road towards Puerto de Pajares 06.VI.2010 *B. Estébanez 3785*; **USA. Montana: [2]** Stillwater Co. 45º32'N 109º49'W *Vitt Ecx. 43* (DUKE) **[1]** 562OR (CONN); ***Orthotrichum bartramii*. MEXICO. Baja California Sur: [2]** Todos los Santos 23º33'05,4"N 109º59'07,5"W 20.VIII.2010 *F. Lara & E. San Miguel*; **Puebla: [3]** Iztapopo N.P. Buenavista 19º05'35,8"N 98º36'25,0"W 5.VIII.2010 *F. Lara*; **U.S.A. Arizona: [1]** Cochise Co. Chiricahua Mts. 31º56'06"N 109º16'29"W 28.III.2007 *Kellman 5457*; ***Orthotrichum casasianum*. SPAIN. Álava:** Luquiano 25.I.1997 *B. Albertos et al.*; ***Orthotrichum cupulatum*. AUSTRALIA. New South Wales: [5]** 8.X.1988

H. Streimann 39642 (CBG-8805222); **CANADA. British Columbia: [4]** 49:25 N119:40W 9.VI.1980 *T.T. McIntosh 4253 & A. Kruckeberg*; **GERMANY. Baden-Würtenberg: [1]** 8.I.1992 *Sauer* (CONN); **[2]** 25.VI.1990 *Sauer* (CONN); **[3]** 25.VI.1990 *Sauer* (CONN); ***Orthotrichum flowersii*. U.S.A. Nevada:** Nye Co. Death Valley Nat. Park. Grapevine Mts. 36º58'03,2"N 117º05'18,4"W 18.I.2003 *J.Shevock 23691* (CAS-1045756); ***Orthotrichum patens*. GERMANY. Baden-Würtenberg:** 24.IX.1990 *Sauer* (CONN); ***Orthotrichum pilosissimum*. U.S.A. Nevada: [4]** Eureka Co. east of Monitor Valley 39º18'04"N 116º31'01"W 31.V.2002 *J. Shevock 22368* (CAS-1039515); Humboldt Co. **[3]** Santa Rosa Mts. 41º28'34"N 117º44'49"W 29.X.2008 *R. Garilleti et al.*; **[1]** Mineral Co. Anchorite Hills 38º13'31"N 118º39'11"W 28.X.2008 *F. Lara et al.*; **[2]** Nye Co. Alta Toquima Wilderness 38º47'36"N 116º51'29"W 31.V.2002 *J.R.Shevock 22397* (CAS- 1040002); ***Orthotrichum pulchellum*. CANADA. British Columbia: [1]** Victoria, Beacon Hill Park 3.XII.2001 *F. Lara*; **[2,3**] Campus of the University of Victoria 5.XII.2001 *F. Lara*; **SPAIN. Álava: [4]** Luquiano 21.VI.2010 *R. Medina*; ***Orthotrichum pumilum*. ESTONIA.** L-Vinemaa 25.V.1991 *M. Leis* (TU-170); ***Orthotrichum scanicum*. GREECE. Sterea Hellada: [1**] Between Brallos and Iti 38º44'N 22º25'E 27.VII.1999 *F. Lara, V. Mazimpaka & M. J. Cano*; **SPAIN. Guadalajara: [2]** Chequilla 4.VI.2004 *L. Pokorny*; ***Orthotrichum schimperi*. ITALY. Sicily. [3]** Mt. Nebrodi 8.VIII.2011 *LoGiudice*; **ROMANIA. Maramureş: [1]** Rodna Mts. 16.VIII.2003 *F. Lara*; **TUNISIA. [2]** Aïn-Draham 36º46'32"N 08º41'33"E 22.III.2005 *F. Lara & E. San Miguel*; **U.S.A. California: San Diego Co. [4]** Cuyamaca Rancho S. P. 32º42'03"N 116º34'44"W 15.XI.2008 *F. Lara, R. Garilleti & J. Shevock*; ***Orthotrichum sharpii*. MEXICO. Veracruz:** La Joya 19º37'08.4"N 97º02'41,1"W 8.VIII.2010 *F. Lara*; ***Orthotrichum stramineum*. SPAIN. La Rioja: [2]** Valvanera 15.V.1990 *E. Fuertes* (H); **GERMANY. Baden-Würtenberg: [1]** 24.IV.1993 *Sauer* (CONN); ***Orthotrichum underwoodii*. U.S.A. California:** Alameda Co. Wilcat Canyon 20.IX.2008 *F. Lara, R. Garilleti & D. H. Norris*; ***Nyholmiella obtusifolia*. FRANCE. Haute-Savoie: [2]** Chamonix VII.2009 *B. Estébanez 3534*; **SPAIN. Burgos: [1]** Covanera 42º44'9.66"N 3º48'5.25" W 5.XII.2010 *F. Lara*; ***Ulota crispa*. TURKEY. Trabzon:** path near Uzungöl 40º35'15'N 40º20'29"E 9.VII.2005 *F. Lara, R. Medina & V. Mazimpaka*;

IV.5

Orthotrichum tenellum

IV.5 Unnoticed diversity within the disjunct moss *Orthotrichum tenellum* (Orthotrichaceae) validated by morphological and molecular approaches

IV.5 Los estudios morfológicos y moleculares validan una diversidad inadvertida en el musgo de distribución disyunta *Orthotrichum tenellum* (Orthotrichaceae)

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Artículo inédito

Abstract

Transcontinental disjunctions in bryophytes are common and constitute a field of intense research due to their diverse underlying causes. Within these disjunctions, a robust species delimitation is needed in order to avoid biased biogeographic conclusions. We addressed the case study of the disjunct moss *Orthotrichum tenellum*, which up to now has been considered to be present in Western North America and the Western Old World. We conducted a deep morphological revision together with molecular analyses using four plastid loci (*atpB-rbcL, rps4, trnG* and *trnL-F*) and two nuclear ones (*AdK* and *ort-Lfy*). Instead of a single disjunct taxon or two vicariant taxa, all the methods congruently revealed the existence of six well-supported species. All of them are morphologically diagnosable and none of them shows any transcontinental disjunction. Two of these species are restricted to the Old World (*O. tenellum* s. s. and *O. comosum* sp. nov.) and the remaining four are American endemics (*O. coulteri*, *O. norrisii*, *O cucullatum* sp. nov. and *O. franciscanum* sp. nov.).

Resumen

Las disyunciones transcontinentales en briófitos son frecuentes y constituyen un campo de investigación activa debido a las diversas causas que las provocan. En el marco de estas disyunciones, se hace necesaria una delimitación de especies robusta para evitar sesgos en las conclusiones biogeográficas. En el presente estudio se aborda el caso del musgo disyunto *Orthotrichum tenellum*, que hasta la fecha se ha considerado presente en el oeste de Norteamérica y el occidente del Viejo Mundo. Se ha llevado a cabo una profunda revisión morfológica junto con análisis filogenéticos empleando cuatro regiones del genoma cloroplástico (*atpB-rbcL, rps4, trnG* and *trnL-F*) y dos del nuclear (*AdK* and *ort-Lfy*). En lugar de un único taxón disyunto o de dos especies vicariantes, ambos métodos revelaron de forma congruente la existencia de seis especies bien definidas. Todas ellas son identificables morfológicamente y ninguna muestra una distribución disyunta. Dos de estas especies están restringidas al Viejo Mundo (*O. tenellum* s.s. y *O. comosum* sp. nov.) mientras que las restantes son endemismos americanos (*O. coulteri, O. norrisii, O cucullatum* sp. nov. y *O. franciscanum* sp. nov.).

Introduction

Species delimitation still represents a significant challenge in the modern Life Sciences, with consequences on both Conservation Biology and the understanding of evolutionary processes. In recent times, discussions on this issue are increasingly gaining prominence (Sites Jr & Marshall, 2003; Wiens, 2007). In this context, the approaches of integrative taxonomy (Dayrat, 2005) that seek a reciprocal illumination (Steele & Pires, 2011) among different sources of information (e.g. morphological and molecular data) provide several advantages. Namely, integrative taxonomy not only results into more robust and trustworthy species delimitations than partial approaches, it also makes the biodiversity accessible and diagnosable by a higher number of researchers.

The study of organisms that are "morphologically austere", where a substantial amount of cryptic diversity may remain still undiscovered (Bickford et al., 2007), can be especially benefited by integrative approaches. Bryophytes belong to this kind of organisms, since their relatively simple organization results in a limited availability of morphological characters. In the past, many species were described on the basis of different distribution areas (Shaw, 2001). However, a more recent interpretation assumes that transcontinental disjunctions are common among bryophytes. There are indeed evidences pointing out that oceans frequently do not constitute a reproductive barrier (Heinrichs et al., 2009). The contribution of the molecular phylogenetic approach to disjunct bryophyte taxonomy has yielded a reduction of species number as a general trend (Vanderpoorten & Shaw, 2010; but see Medina et al., 2011). Other cases showed a complex genetic structure that suggested the existence of several cryptic species (Shaw, 2001; Heinrichs et al., 2009), that is, species that are indistinguishable by comparative morphology. This has also been reported within a specific geographical area (e.g. Fernandez et al., 2006; Wachowiak et al., 2007).

Most of the studies that conclude the existence of cryptic bryophyte species lack a critical morphological evaluation of the studied specimens and the cryptic nature of the diversity discovered by molecular data is assumed. However, there are precedents of successful works where integrative taxonomical conclusions are reached (Renner et al., 2010; chapter IV.4), and the specialized taxonomical contribution became essential for the resolution of the problems.

We present here a new case study on the diversity of the moss genus *Orthotrichum* Hedw. With more than 155 species (Goffinet et al., 2007), it is one of the most diverse among bryophytes. The target of this work is *Orthotrichum tenellum* Bruch ex Brid., a taxon that has a long and complex taxonomical and nomenclatural history. *Orthotrichum tenellum* has been interpreted as a single, very variable species following a transcontinental disjunction between the Western Old World and Western North America. This distribution area is relatively common among bryophytes (Schofield, 1988), and spans two of the biodiversity hotspots of the World: the Mediterranean Basin and the Floristic Californian Region (Myers et al., 2000; Mittermeier et al., 2005). During the revision of North American *Orthotrichum* specimens, a notorious incongruence between the morphological circumscription of *O. tenellum* in Europe and America was detected.

Besides the description of the vicariant *Orthotrichum norrisii* F. Lara, R. Medina & Garilleti (Medina et al., 2008), a significant part of the diversity within *O. tenellum* remained undefined. In order to resolve this, a morphological revision of *O. tenellum* s.l. was undertaken and its results tested with molecular data. We present here the results of this research, which resolve the taxonomical questions and provide a new biogeographic interpretation.

Material and methods

Taxon sampling and morphological studies. More than 550 specimens formerly identified as *O. tenellum* were examined. Herbarium vouchers, including types, belong to different collections: B, CAS, COLO, FH, MICH, NY, UC and authors' herbarium at Universidad Autónoma de Madrid (duplicates deposited at VAL). Other specimens were materials recently collected by us in different field campaigns or donated by other collectors. A selection of representative specimens is listed in the Appendix.

The first step for species delimitation consisted in a preliminary clustering of the specimens into species candidates according to a deep morphological study. The morphological characters used for this purpose included most of those previously considered valuable for *Orthotrichum* taxonomy (Lewinsky, 1993), plus some other diagnostic features established for this work on the basis of the experience of the research group on the genus. Relevant traits of the gametophyte included plant size and habit, several leaf characters such as shape, margins and apical differentiations, gemmae morphology as well as calyptrae and vaginulae shape, size and hairiness. The sporophyte provides many diagnostic characters, and sterile specimens are often impossible to identify safely. Sporophyte characters span seta length, capsule length and shape, constitution of exothecial bands, stomata position and degree of covering, configuration and ornamentation of peristome and spore size.

DNA extraction and sequencing. A selection of specimens was used for the performance of phylogenetic analyses. Among this set of specimens is included a representative sample of all the species candidates formerly established in the morphological approach. The identification of these specimens was carefully double-checked before the DNA extraction. Other specimens from several *Orthotrichum* taxa with immersed stomata (subgenera *Pulchella* and *Orthotrichum* sensu Lewinsky, 1993) were included in the selection in order to outline the phylogenetic relationships among species and some close lineages. For the same purpose, this selection also included several taxa especially diversified in Western North America. Some additional specimens of *Orthotrichum* with superficial stomata and other Orthotrichaceae were also included as outgroup. Finally, 104 specimens were considered for the analyses of the chloroplast loci and 76 specimens were considered in the analyses of the nuclear *loci*. Genomic DNA from the specimens was extracted following the CTAB extraction protocol (Doyle, 1987) modified for Orthotrichaceae as described in Goffinet et al. (1998). The DNA of some particular specimens was extracted with a commercial kit (DNeasy® Plant Mini Kit; Qiagen) following the manufacturer's instructions.

After testing the variability of several genomic regions, six loci were targeted; four within the chloroplast genome (*atpB-rbcL, rps4, trnG* and *trnL-F*) and two from the nuclear genome (*AdK* and *ort-Leafy*). The chloroplast loci were amplified by simple PCR in a final volume of 25 µL with 0.2 µL Taq DNA Polymerase (for *loci atpB-rbcL* and *trnG*) or HotMaster Taq DNA Polymerase (for *loci rps4* and *trnL-F*) (5 PRIME), 2.5 µL of the Mg^{2+} Buffer provided by the manufacturer, 1 µL of 10µM dNTP mix, 1 µL of each primer (10 µM) and 1 µL of the DNA extract. After an initial denaturation step of 5 min at 94 ºC, 30 cycles were carried out consisting of 30 s denaturation at 95º C, 1 min of annealing (48 ºC for *trnG* and 52 ºC for the other *loci*) and a 30 s extension at 68º C, followed by a final extension step of 7 min.

The nuclear *loci* were amplified by nested PCR. The external reactions were carried out in the same conditions of reactive volumes and concentrations as those for the chloroplast *loci*, but programming an annealing temperature of 50ºC. For the internal PCR, 0.5 µL of the product of the external PCR was used as the template and the annealing temperature was set to 54ºC. All the primers used for the PCR reactions are listed in Table IV.5.1 with their corresponding bibliographic source, except for internal primers of nuclear *loci*, which were designed for this work.

PCR products were purified using the Nucleospin Extraction Kit (Machery-Nagel). Sequencing reactions were conducted in a final volume of 10 µL with ABI BigDye Terminator and cleaned as described in Buck et al. (2000). The samples were read using an ABI Prism 3100 Genetic Analyser with the same primers used in the PCR reactions.

Phylogenetic analyses. Sequence contigs were assembled using Sequencher 3.1.1. (Machery-Nagel) and Geneious 5.1.7.0 (Biomatters Ltd.) and the matrices for each locus were built using PhyDE® (Müller et al., 2006). The sequences were first aligned with

Clustal W (Larkin et al., 2007) or, for those loci with higher alignment difficulties, with MUSCLE (Edgar, 2004), then adjusted and trimmed by the ends manually. The compatibility among the different loci of each genome was checked by the ILD test (Farris & al., 1994) based on 1,000 replications with TBR selected as swapping algorithm. The partial alignments of the different loci for each genome were combined into two matrices (chloroplastic and nuclear). The indels of these matrices were codified as an additional adjacent block for each matrix according to the simple-coding algorithm (Simmons & Ochoterena, 2000) implemented in SeqState 1.4 (Muller, 2005).

The chloroplast and nuclear matrices were analyzed under Maximum Likelihood (ML) and Bayesian Inference (IB). For these analyses, the chloroplast and nuclear matrices were partitioned according to the different loci that integrate them, plus a final partition for the codified indels. The best substitution model for each partition was estimated using the Akaike Information Criterion (AIC) and the Bayesian Information Criterion (BIC) as implemented in jModelTest 0.1.1 (Posada, 2008). When both criteria did not agree in the best model, replicates of all the analyses were conducted to check that the model choice did not result in significantly different results. The model with the lowest number of variables for each locus was chosen for the final analyses. For the indel partition in both matrices, the F81 model was implemented in ML and IB analyses, as recommended in Ronquist et al. (2005).

For the ML method, the matrices were analyzed with the software GARLI 2.0 (Zwickl, 2006) implementing a partitioned analysis, specifying the substitution model for each partition and using the default settings for the remaining parameters. Nonparametric bootstrapping (Felsenstein, 1985) with 200 pseudoreplicates was used for evaluating the statistical support of the branches, and the consensus tree was obtained with the program SumTrees 3.3.1 as implemented in DendroPy 3.8.0 (Sukumaran & Holder, 2010).

Bayesian Inference phylogenetic analyses were carried out using MrBayes 3.2.1 (Ronquist & Huelsenbeck, 2003) running a partitioned analysis and specifying the substitution model for each block. The Markov Chain MonteCarlo (MCMC) simulation was executed for 5,000,000 generations for the chloroplast genome matrix and 2,000,000 generations for the nuclear genome matrix, both with two runs and four chains, sampling trees and parameters each 1,000 generations. The 50% majority-rule consensus tree was obtained for each analysis after burning 25% of the starting trees and plotted using FigTree 1.3.1 (Rambaut & Drummond, 2010).

The species delimitation was additionally tested using the genealogical sorting index (gsi_T; Cummings et al., 2008). For a given rooted phylogeny, this method estimates the degree of exclusive ancestry of the groups manually labeled, in this case, according to the morphological hypothetic delimitation. The gsi_T maximum value is 1, acting as a

	locus		Primer pair	Sequence 5'-3'	Annealing temperature	Source	Best model (jModelTest)	
							AIC	BIC
chloroplast	*atpB-rbcL*		*atB*	ACATCKARTACKGGACCAATAA	52 ºC	Chiang et al., 1998	GTR+G	GTR+G
			rbcL	AACACCAGCTTTRAATCCAA				
	rps4		*rpsA*	ATGTCCCGTTATCGAGGACCT	52 ºC	Nadot et al., 1994	GTR+G+I	GTR+G+I
			trnaS	TACCGAGGGTTCGAATC				
	trnG		*trnG-F*	CGGGTACGGGAATCGAAC	48 ºC	Souza-Chies et al., 1997	GTR+G	HKY+G*
			trnG-R	GCGGGTATAGTTTAGTGG				
	trnL-F		*trnC*	ATTTGAACTGGTGACACGAG	52 ºC	Pacak & Szweykowska-Kulinska, 2000	GTR+G	HKY+G*
			trnF	CGAAATCGGTAGACGCTACG				
nuclear	*AdK*	ext	*AdK-F*	GAAGAAGCCAGAAAACTGGGC	50 ºC	McDaniel et al., 2010	GTR+G	HKY+G*
			AdK-R	GTCACCTTTCCATCTTCAGCAAC				
		int	*Ort-AdK-F*	GTGGAGAGGGCGAAG	54 ºC	Designed for this study		
			Ort-AdK-R	ACCTGGGCAAATGTC				
	Ort-Leafy	ext	*LFY1428F*	GGAAGAAGAATGGATTGGACTA	50 ºC	Shaw et al., 2003	GTR+G	HKY+G*
			LFY2327R	TCTCATCTTTGGCTTGTTTATG				
		int	*Ort-Lfy-F*	CTSTTTGATTTGTATGAACAGTG	54 ºC	Designed for this study		
			Ort-Lfy-R	GTAGCYAGCYCCTGTMAGTTTCG				

Table IV.5.1. Primers used in the Polymerase Chain Reaction (PCR), optimal annealing temperature for each locus, bibliographic reference and best substitution model for each locus according the AIC and BIC criteria implemented in jModelTest. When different models were obtained for the same locus, alternative analyses with all the possible model combinations were conducted to check that the model choice did not result in significantly different results. In those cases, the model with fewer variables was chosen for the final analysis (marked with an asterisk) monophyly test in which the null hypothesis to be rejected is that the labeled clusters are random. Coalescent simulations for 1,000 generations were used for the resulting collection of bootstrap pseudorreplicates trees for (ML) and postburn-in trees (IB). The method also provides a P-value for the probability that a random mating could result into the same clustering.

Results

Morphological approach. The taxonomical revision of the studied materials reveals that within the prevailing idea of a very variable and disjunct species, *Orthotrichum tenellum* s.l. is integrated by six different morphotypes (Table IV.5.2). All these morphotypes correspond to distinct taxa, each of them supported by a consistent combination of characters. *Orthotrichum tenellum* s.s. seems to be absent from the American continent. Instead, four different taxa are present in the Californian Region: *Orthotrichum coulter*i Mitt., a former synonym reinstated here as a valid species (28% of the examined American specimens); *Orthotrichum norrisii* F. Lara, R. Medina & Garilleti (27%) and two new species: *Orthotrichum cucullatum sp. nov.* (25%) and *Orthotrichum franciscanum sp. nov.* (10%). Interestingly, among the remaining studied specimens, a marginal (<10%) but recurrent number of misidentifications correspond to *Orthotrichum columbicum* Mitt. and similar species (*Orthotrichum consimile* Mitt., *O. confusum* R. Medina, F. Lara & Garilleti and *O. persimile* F. Lara, R. Medina & Garilleti) (chapter IV.4). Conversely, none of these taxa is present in the Old World, where in addition to *O. tenellum* s.s., another minority form was found in the course of this revision: *Orthotrichum comosum* sp. nov. This morphological species delimitation was used as a working hypothesis for the molecular phylogenetic analyses.

Phylogenetic reconstruction: cloroplastic *loci*. The combined alignment of the four chloroplast *loci* plus the indel block consists of 2432 base pairs (*atpb-rbcL 569* bp; *rps4 651* bp; *trnG 538* bp; *trnL-F 532* bp; indel block 142 bp), of which 402 (16.5%) are parsimony-informative. The ILD test did not find significant incongruence among the combination of loci except for *atpB-rbcL* versus *rps4* and *trnG*. The results of the individual analysis of each locus were manually checked to confirm that there were no conflicting clades at the species delimitation level. The results of the ML and IB analyses (Fig IV.5.1) resolve all the morphological species candidates into monophyletic groups. The main ingroup clades of the whole analysis are arbitrarily named A-D.

Orthotrichum tenellum and *O. norrisii* are resolved as two closely related sister clades, each one with a Posterior Probability value of 1.0 and 0.72 according to the BI analysis. ML bootstrap support values are low (57 and 55), but gsi_T values are over 0.8 for both species

	O. tenellum	***O. coulteri***	***O. norrisii***	***O. comosum***	***O. cucullatum***	***O. franciscanum***
Plants size	0.3–1.2 cm	0.3–0.8(–1.4) cm	0.6–1.0(–1.5) cm	0.3–0.9 cm	0.3–0.8(–1.2) cm	(0.2–)0.4–0.8 cm
Leaf shape	Ligulate to ovate-lanceolate	Long triangular to ovate-lanceolate	Lanceolate to ovate-lanceolate	Linear-lanceolate to ovate-lanceolate	Ligulate to ovate-ligulate	Linear lanceolate to ovate-ligulate
Leaf apex	Rounded to acute; channelled	Acute to subobtuse, blunt	Acute to rounded; folded or channelled	Blunt-acute to acuminate; mucronate	Blunt to rounded; almost plane or cuculate	Acute to acuminate; plane, with hyaline apiculum
Leaf margin	Recurved	Recurved	Recurved	Recurved (revolute)	Recurved	Distinctly revolute at mid-leaf
Vaginula hairiness and length	Naked; 0.3–0.6 mm	With abundant hairs; (0.5–)0.6–0.8 mm	Usually with hairs; 0.3–0.8 mm	Naked; 0.25–0.4(–0.6) mm	Hairy (exceptionally naked); 0.2–0.3 (–0.5) mm	Sparsely hairy; 0.3–0.5 mm
Calyptra shape and hairiness	Fusiform to oblong-conic, sparsely hairy	Narrowly oblong-conic to fusiform, densely hairy	Oblong-conic, hairy towards apex	Oblong-conic, with a distinct apical comma	Ovoid to oblong-conic, naked or with sparse hairs	Oblong-conic to fusiform, sparsely hairy
Seta length	0.4–0.8(–1.0) mm	0.8–1.5(–2.2) mm	(0.5–)0.7–1.0 mm	0.3–0.6 mm	(0.35–)0.5–0.75 mm	0.4–0.8(–1.0) mm
Mature and empty capsule shape	Subcylindrical, constricted below mouth	Cylindrical, slightly constricted below mouth	Cylindrical, not constricted	Subcylindrical, constricted in the upper half	Subcylindrical, constricted in the upper half	Subcylindrical, variably constricted in the upper half
Exothecial bands width	4–6 cells	(2–)3–4 cells	2(–3) cells	4–5 cells	4–6 cells	3–4(–5)
Stomata position	Lower half, but not the neck	Lower third and neck	Lower half, but not the neck	Lower half and neck	Lower half, but not the neck	Lower half and neck
Exostome OPL ornamentation	Dense papillae (Exceptionally sparse isolated striae)	Dense papillae (ocassionally with vermicular lines towards apex)	Dense papillae, often with vermicular lines towards apex	Basal papillae and vermicular lines towards apex	Basal papillae and vermicular lines towards apex	Papillae and faint transversal lines at base, vermicular lines towards apex
Spore size	12–17(–21) µm	12–18(–22) µm	(12–)15–17(–21) µm	(10–)12–16(–18) µm	12–16(–20) µm	(10–)12–16(–20) µm

TABLE IV.5.2. Comparative chart of the morphological characters that allow the diagnosis of the species studied in this work.

with P=0.001. This *tenellum+norrisii* clade is grouped together with another clade including *O. bartramii* R. S. Williams, *O. sharpii* H. Rob. and *O. comosum*, also with high support of PP, Bootstrap and gsi_T values. Clade A, (including all these species plus some others belonging to subgenus *Pulchella*), shows weak support in this analysis.

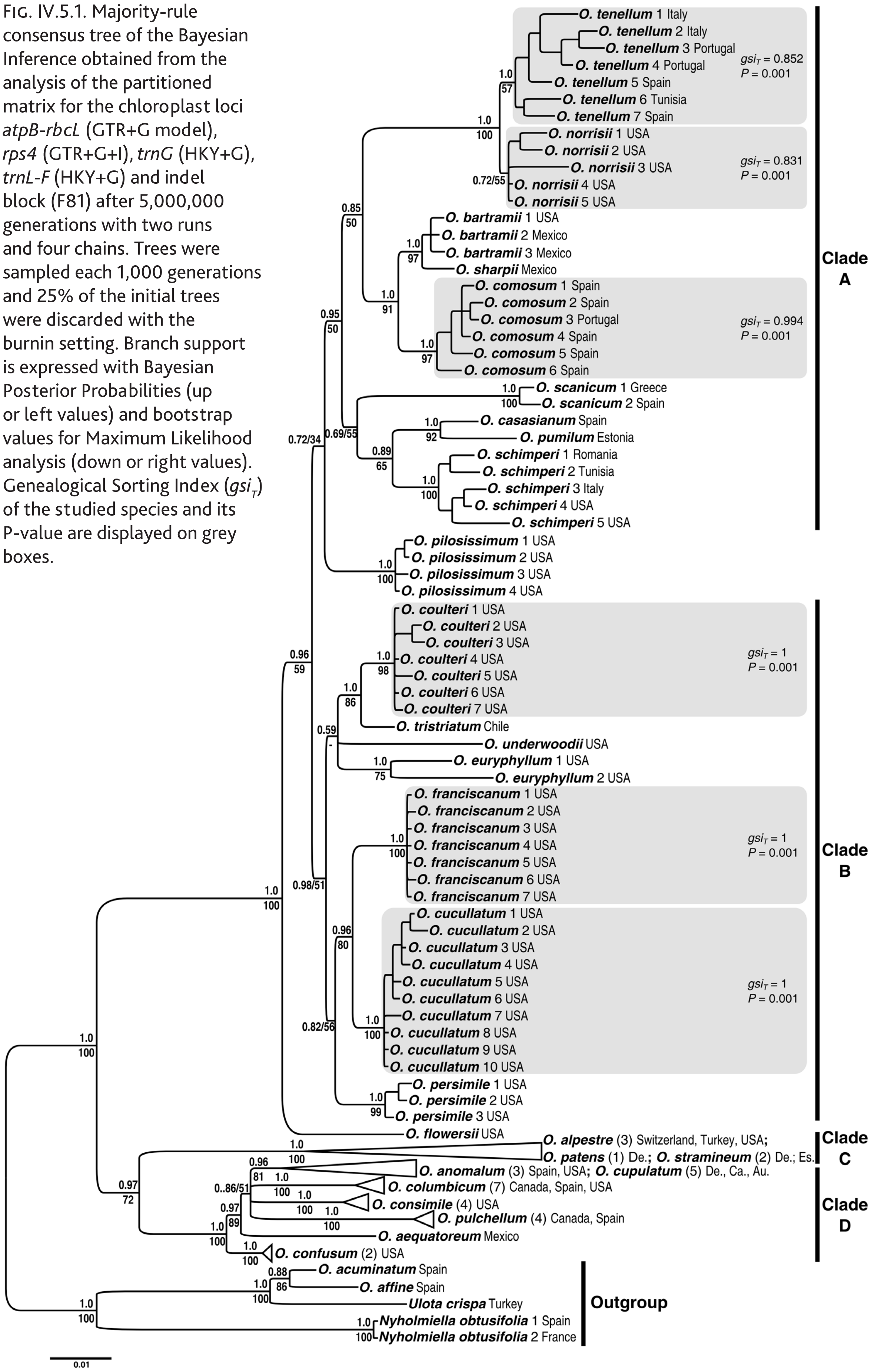
The other tested species, *O. coulteri*, *O. cucullatum* and *O. franciscanum*, are also resolved as clades with high PP and bootstrap values, as well as gsi_T=1 with P=0.001. They also seem to be closely related to other *Orthotrichum* endemics from the Californian Region: *O. persimile*, *O. euryphyllum* Venturi in Röll, and *O. underwoodii* F. Lara, Garilleti & Mazimpaka, as well as the Andean *O. tristriatum* Lewinsky. This "Western clade" (Clade B), together with clade A is clustered with *O. flowersii* Vitt into a well supported monophyletic group.

The remaining ingroup specimens are resolved into two basal clades (C and D, collapsed in fig. IV.5.1) Clade C contains *Orthotrichum alpestre* Hornsch. ex B.S.G., *O. patens* Bruch ex Brid. and *O. stramineum* Hornsch. For its part, clade D hosts some representatives of subgenus *Orthotrichum* (*O. anomalum* Hedw. and *O. cupulatum* Hoffm. ex Brid.) and other *Pulchella* taxa (*O. consimile* Mitt., *O. columbicum* Mitt., *O. pulchellum* Brunt., *O. aequatoreum* Mitt. and *O. confusum* R. Medina, F. Lara & Garilleti). Both, C and D, are strongly supported by Bayesian PP, ML Bootstrap and gsi_T values. The topology of this tree has some nodes with low ML bootstrap values at several basal dichotomies, but they do not affect the species delimitation.

Phylogenetic reconstruction: nuclear *loci*. The sequences obtained when the *leafy* nuclear *locus* was targeted turned out to be an anonymous region with no matches at NCBI BLAST. These "*ort-leafy*" sequences are very variable within the ingroup (from 800 to 1100 bp) due to very long indels. However, all the sequences kept several common homologous domains, including the beginning and the end, and could be aligned. Preliminary analyses of this *locus* with and without indel codification showed results consistent with those of *AdK* and the chloroplast loci, and the ILD test did not find any significant incongruence between them. Consequently, *ort-leafy* region was considered informative and included in the analyses.

The combined alignment for the *AdK* and *ort-leafy loci* contains 73 taxa and 3181 base pairs (*AdK* 465 bp; *ort-leafy* 2594 bp; indel block 122 bp), 506 of which (15.9%) are parsimony-informative. The resulting consensus topology of the ML and IB analyses (Fig. IV.5.2) also resolves all the species candidates as reciprocally monophyletic groups with very high values of Bayesian PP, ML bootstrap and gsi_T with P=0.001 (including *Orthotrichum tenellum* and *O. norrisii*). Most of the remaining nodes show also robust supports for ML and IB.

Fig. IV.5.1. Majority-rule consensus tree of the Bayesian Inference obtained from the analysis of the partitioned matrix for the chloroplast loci *atpB-rbcL* (GTR+G model), *rps4* (GTR+G+I), *trnG* (HKY+G), *trnL-F* (HKY+G) and indel block (F81) after 5,000,000 generations with two runs and four chains. Trees were sampled each 1,000 generations and 25% of the initial trees were discarded with the burnin setting. Branch support is expressed with Bayesian Posterior Probabilities (up or left values) and bootstrap values for Maximum Likelihood analysis (down or right values). Genealogical Sorting Index (gsi_T) of the studied species and its P-value are displayed on grey boxes.



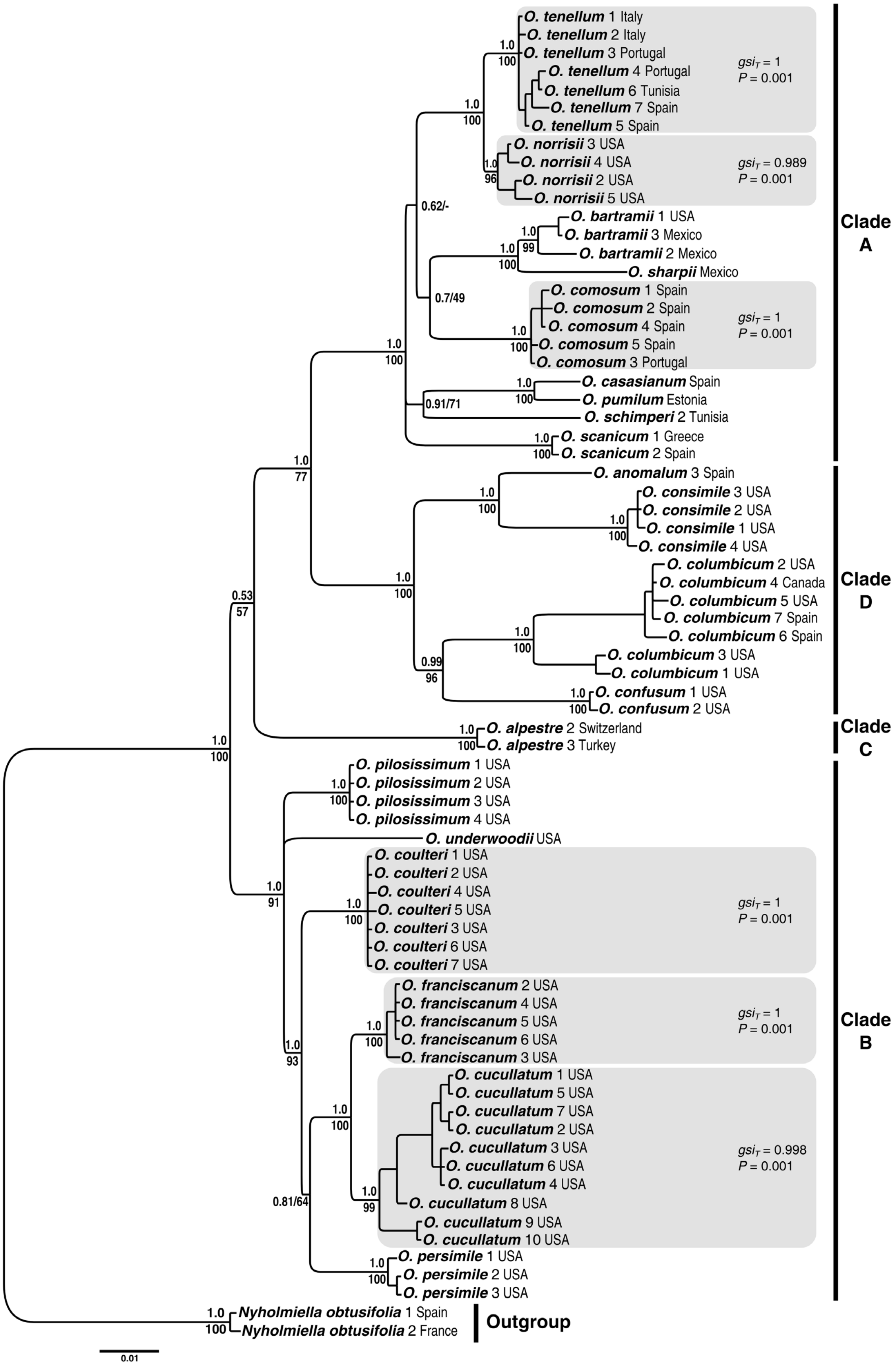


FIG. IV.5.2. Majority-rule consensus tree of the Bayesian Inference obtained from the analysis of the partitioned matrix for the nuclear loci *AdK* (HKY+G model), *ort-Lfy* (HKY+G) and indel block (F81) after 2,000,000 generations with two runs and four chains. Trees were sampled each 1,000 generations and 25% of the initial trees were discarded with the burnin setting. Branch support is expressed with Bayesian Posterior Probabilities (up or left values) and bootstrap values for Maximum Likelihood analysis (down or right values). Genealogical Sorting Index (*gsi*$_T$) of the studied species and its P-value are displayed on grey boxes.

The composition of clades A-D is equivalent to that from the chloroplast analysis tree (with the only exception of *O. pilosissimum*, that is resolved in clade B instead of clade A), and all of them show very high PP and bootstrap supports. The topological relationships among clades A-D, however, differ strongly from that of the chloroplast tree. Clades A and D are sister groups according to the nuclear results, and A+D is sister group of *O. alpestre* (representative of clade C), while clade B shows the most basal position.

Discussion

This study aimed to disentangle the insights of the disjunct, morphologically variable moss, *Orthotrichum tenellum*. The results reveal a hidden diversity lumped within the wide and generally accepted concept of this moss. Instead of a single, disjunct taxon, six different species can be identified: two of them are from the Old World (*O. tenellum* s.s. and *O. comosum*), while the remaining four are restricted to the Californian region (*O. coulteri, O. norrisii, O. cucullatum* and *O. franciscanum*). All of them can be diagnosed by unique combinations of morphological characters and constitute well supported clades according to the phylogenetic analyses. In addition, the two topologies resulting from these analyses, although differing in some aspects, suggest that the species belong to different lineages and thus have undergone separate evolutionary histories.

Morphological delimitation. The correct identification of the studied *Orthotrichum* species can be a thorny issue, and not by chance they have been historically overlooked. They share certain characters that may have contributed to consider them a single disjunct, taxon: 1) small to medium-sized mosses; 2) mostly lanceolate to ovate-lanceolate leaves, appressed when dry and with recurved margins; 3) Capsule immersed to emergent (except for *O. coulteri*) and ribbed; 4) Stomata cryptopore in the lower half of the urn, almost to completely covered by surrounding cells and 5) a similar peristome configuration of 8 recurved exostome teeth alternate with 8 endostome segments. Despite these similarities, they can be segregated by morphological characters. The most useful diagnostic characters within this species group are leaf apex differentiations, vaginula and calyptra hairiness, capsule shape, width of exothecial bands and stomata position (Table IV.5.2).

Orthotrichum tenellum can be recognized by its ovate-lanceolate leaves and distinct apices with incurved margins, most frequently forming a short channel. This species is also characterized by a naked vaginula and a slightly hairy calyptra. Its emergent capsule is subcylindrical, constricted below the mouth, and markedly ribbed, being the exothecial bands constituted by 4-6 rows of cells. The stomata are placed in the lower half of the urn, but not reaching the neck. Finally, the peristome is formed by an exostome of 8 pairs of teeth that alternate with 8 endostome segments.

The most similar species is *O. norrisii*, which shares with *O. tenellum* the leaves with channeled apices, an emergent, ribbed capsule, the stomata placed in the lower urn but absent from the neck and a similarly developed peristome. Unlike *O. tenellum, O. norrisii* typically has hairy vaginulae and cylindrical capsules that are not constricted below the mouth when mature and empty. Additionally, exothecial bands of *O. norrisii* are distinctively 2 cells wide, while *O. tenellum* bands are wider, typically 4-cell wide (Medina et al., 2008). The Outer Peristome Layer (OPL) of the exostome is densely papillose in both taxa, but only in *O. norrisii* it is also commonly ornamented by vermicular lines towards the apex.

The channeled apex is a very distinctive trait that is also shared by other *Orthotrichum* species, namely *O. bartramii*, *O. scanicum* Grönvall, *O. pilosissimum* and *O. columbicum*. Several morphological characters prevent confusions with *O. tenellum* or *O. norrisii*. Leaves of *O. bartramii* are oblong and abruptly cuspidate into a well formed apical channel, and the rhizoids are typically ascending along the stems. Additionally, the capsules are oblong to oblong-cylindrical, constricted below the mouth and with exothecial bands of 4-8 cells wide (Vitt, 1973). *Orthotrichum scanicum*, like *O. norrisii*, has narrow exothecial bands, but its splitting exostome teeth, endostome of 16 segments and characteristic convex, orange operculum clearly distinguish this species (Medina et al., 2009). For its part, *O. pilosissimum* is easily identified by its remarkably long and branched axillary hairs, as well as by its strongly constricted capsules (Medina et al., 2011). Finally, *O.columbicum* is clearly distinct from the former mosses for its flexuose in dry and long triangular leaves, its completely exserted capsules and naked calyptrae and vaginulae (chapter IV.4).

Another species that strongly resembles *O. tenellum* is *O. comosum*. The most distinctive trait of this small sized moss is the oblong-conic calyptra, with a terminal comma of papillose hairs. The leaves show an acute to acuminate apex, often mucronate, but never channeled. The vaginula is naked, the capsule is immersed, markedly constricted and strongly ribbed when empty and dry, the exothecial bands are made of 4-5 rows of cells and the stomata are placed in the lower half of the urn and neck. Up till now, this species has been overlooked and identified as other European and Mediterranean species, mainly *O. tenellum* and *O. alpestre*. Unlike *O. tenellum*, this new moss has leaves without channeled apices and its stomata are present in the neck. In addition, the outer surface of the exostome teeth has apical striae in *O. comosum*, which are very rare in *O. tenellum*. Regarding the differences between *O. alpestre* and *O. comosum*, the latter can be distinguished by its completely naked vaginula (hairy in *O. alpestre*) and frequently propaguliferous leaves (rare and scarce in *O. alpestre*). In addition, *O. alpestre* has constantly long, branched papillae on the mid-leaf cells, a character that has never been observed in *O. comosum*. These two species also have very different ecological preferences: while *O. comosum* is a typically Mediterranean taxon, *O. alpestre* preferscolder and mountainous habitats.

Orthotrichum franciscanum can be identified by its distinct leaves: linear-lanceolate to ovate-ligulate, with distinctly revolute margins towards the middle of the lamina and apices acuminate, not channeled, often ended in one or a few elongated hyaline cells. This species has sparsely hairy or naked vaginulae and sparsely hairy calyptrae, subcylindrical and furrowed capsule with 3 to 5 cells wide exothecial bands and stomata in the lower half of the urn, including the neck. Exostome teeth pairs are sometimes truncated in *O. franciscanum*, a character that may also help in the identification.

Orthotrichum cucullatum also have very distinctive leaves: ligulate to ovate-ligulate with characteristic obtuse-rounded apex, often cucullate and hood-like, but not channeled. This moss bears sparsely hairy vaginulae, while its calyptrae can be naked or with sparse, inconspicuous hairs. *Orthotrichum cucullatum* has subcylindrical, furrowed capsules with 4-6 cells wide exothecial bands and stomata in the lower part of the urn, but absent on the neck.

Finally, *Orthotrichum coulteri* is a species with linear-lanceolate leaves and acute or blunt not-channeled apices. It typically bears densely hairy vaginulae and calyptrae and exserted capsules, which are cylindrical and slightly constricted below the mouth when dry and empty. Exothecial bands are usually 4 cells wide and the stomata are present in the lower urn and neck. Confusions with the previous species are improbable if the mentioned characters are examined. Due to its exserted capsule and hairy calyptra and vaginula, it looks reasonably similar to *O. consimile* and *O. persimile*. These species, however, are best characterized by their slightly flexuose leaves when dry (appressed in *O. coulteri*) and other leaf traits: the hyaline apical cells and plane leaf margins of *O. persimile* and the unguiculate or channeled apices of *O. consimile*.

Phylogenetic reconstruction. *Orthotrichum tenellum* and *O. norrisii* are congruently interpreted as sister clades by the chloroplast and nuclear trees, and their short branch lengths suggest a relatively recent speciation event. These clades are mainly supported by Bayesian PP (1.0 and 0.72 respectively), while ML bootstrap is quite low (57 and 55). This is a common situation in pairs of sibling species that have become reproductively isolated in recent geological times and does not constitute negative evidence against the existence of two species (Shaw, 2009; Vanderpoorten & Shaw, 2010; Zander, 2008). In addition, gsi_T values provide additional evidence for reciprocal monophyly, and nuclear data strongly support this hypothesis too.

All the remaining species candidates are resolved as monophyletic clades with strong Bayesian PP, ML bootstrap and gsi_T values by both chloroplast and nuclear reconstructions. The close relationship of *Orthotrichum comosum* to the clade of *O. bartramii* and *O. sharpii* is reflected in the chloroplast tree, but only weakly supported in the nuclear topology. As for the remaining studied species (*O. coulteri*, *O. cucullatum* and

O. franciscanum), the chloroplast and the nuclear topologies are congruent and suggest that they are closely related.

Concerning the general phylogeny of all the sampled taxa, some affinities can be inferred from the results of the chloroplast and the nuclear analysis. Both trees point towards the existence of four main clades (A-D); however, their relative topology is conflicting for each genome: [(A,B)(C,D)] in the chloroplast tree and [((A,D)C)B] in the nuclear tree. *Orthotrichum flowersii* (only included in the chloroplast tree) would not belong to any of these clades and the position of *O. pilosissimum* is not clear in the chloroplast tree. These kinds of incongruences between the phylogenetic signals of different genomes are relatively frequent in bryophyte studies (e.g. Draper et al., 2007; Hernández-Maqueda et al., 2008). The causes of this incongruence may be diverse and do not constitute an objective of this work, but this potential complexity should be taken into account as a note of caution when the phylogenetic reconstruction of this genus is confronted. In addition, the position in the chloroplast tree of the Orthotrichum species with superficial stomata, *O. affine* and *O. acuminatum*, within the outgroup, supports the hypothesis of *Orthotrichum* s.l. being polyphyletic as already outlined by previous works (Goffinet et al., 2004; Sawicki et al., 2009).

Undoubtedly, the morphological diagnosis of the studied taxa is a complicated but feasible task due to the overall similarity of these species. However, they do not constitute a natural group according to the phylogenetic results. On the contrary, they appear clustered in two different lineages, with separate evolutionary histories. This situation is similar to the one found within the pseudo-cryptic complex of *O. consimile* (chapter IV.4). In both cases, many of the studied morphological characters of *Orthotrichum* have resulted useful in species delimitation. However, they often do not seem to have a straightforward phylogenetic interpretation. For instance, straight-channeled leaf apices are shown by species very distant in the phylogenetic reconstruction, suggesting several independent acquisitions: *O. tenellum*, *O. norrisii*, *O. bartramii*, *O. scanicum*, *O. pilosissimum* and *O. columbicum*. Another example is the hairy vaginulae, which can be found in *O. norrisii*, *O. coulteri*, *O. cucullatum*, *O. franciscanum, O. persimile, O. stramineum, O. patens* and *O. alpestre*. Morphological resemblance is not always correlated with phylogenetic affinity within the ingroup, and the morphological characters may not be useful to outline the phylogeny of this genus beyond the species level. This should also be taken into account in further research.

This study reflects the suitability of an integrative approach to resolve the taxonomy of a conflicting group of taxa (Dayrat, 2005). Although studies on transcontinental disjunctions among bryophytes frequently conclude the existence of a cryptic diversity (Heinrichs et al., 2009), the participation of specialized taxonomists should always be claimed in order to reach, when possible, taxonomical conclusions that make the bryophyte diversity more accessible.

Nomenclature insights. Besides the discussed complexity of the species delimitation, a historical and nomenclatural problem should be also approached in order to understand the causes of the misunderstanding of this group. *Orthotrichum tenellum* was first described from German materials, being the type kept at B! (Bridel, 1826). Some decades later, a new Californian form was described under two different names almost at the same time: *Orthotrichum coulteri* (Mitten, 1864) and *O. cylindrocarpum* Lesq. (Lesquereux, 1865).

The priority of these two names is not a straightforward question and confusions are frequently found in the bibliography about dates of publication. Additionally, some comments after the original description of *O. coulteri* cite literally the protologue of *O. cylindrocarpum* suggesting erroneously that Mitten's name was published later. In fact, *O. coulteri* was published in 1864 (J. Linn. Soc., Bot. Vol. 8 issue 29), while the description of *O. cylindrocarpum* took place in 1865 (Trans. Amer. Philos. Soc. Volume 13, part I). The origin of the confusion may be due to the fact that the oral lecture of Lesquereux's paper (June 19th 1863) preceded Mitten's one (January 22nd 1864), but those both do not constitute dates of valid publication.

In the original publication, Mitten pointed out that *O. coulteri* seemed to be similar to *O. tenellum*, but he also remarked some diagnostic differences from the latter: exserted capsule, more acute leaf apices and more hairy calyptra (Mitten, 1864). The type material (FH!) clearly shows all these traits.

Lesquereux described *O. cylindrocarpum* based on material from Oakland as a species with exserted capsule and densely hairy vaginula (Lesquereux, 1865). We have examined the type materials kept in three different herbaria (FH!, MICH! and NY!) and all these vouchers keep mixed samples. The description of *O. cylindrocarpum* mentions a cladautoicous species with a graceful exserted capsule and a very hairy vaginula, clearly establishing the identity of this taxon within the mixed samples. A careful examination of these materials allowed us to infer Lesquereux's idea of *O. cylindrocarpum*: the only species that is present in all the vouchers and matches the description is *O. coulteri*. The sample of FH is mixed with *O. columbicum*, the sample of MICH with *O. norrisii*, and that at NY with *O. cucullatum*. In the protologue, Lesquereux also mentions that the dry, empty capsule is not contracted below the mouth, but this only applies to the capsules of *O. norrisii* present in the sample of MICH.

In the Manual of the Mosses of North America (Lesquereux & James, 1884), O. coulteri was formally synonymized to *O. cylindrocarpum* without providing the dates of publication. The description therein given is accurate, as well as the plate in the supplement of Icones Muscorum (Sullivant, 1874). *Orthotrichum tenellum* is recorded in the Manual for the first time in America (at the base of the Rocky Mountains, *E. Hall, Wolf*

& Rothrock legit). We have not found this specimen, but considering the overall results of this study, this record should be considered dubious until properly checked.

In The Moss Flora of North America (Grout, 1936), *Orthotrichum coulteri* was combined to *O. tenellum* var. *coulteri* (Mitt) Grout. We have checked some of the exsiccata used for Grout's revision and they correspond to *O. coulteri*. In the description of the Flora, however, a very wide and variable concept of *O. tenellum* was assumed. Grout himself pointed out that the European bryologists did not think that the species described in the Lesquereux & James` Manual was *O. tenellum*, but even though he adopted this oversynthetic criterion. Since Grout's work, this approach has been preferred in North America, and the four species here reported from the area have been synonymized to *O. tenellum* (*O. coulteri*) or overlooked (*O. norrisii*, *O. cucullatum* and *O. franciscanum*). This criterion has spread the idea of *O. tenellum* as a very variable species in America (e.g., Vitt 1973; 2009), however, it does not fit the European populations (Casas et al., 2006; Lara & Cortini Pedrotti, 2001; Smith & Smith, 2004).

Biogeography and ecology. All the studied species live in regions of Mediterranean climate and have their optimum in relatively warm environments, with a dry summer season but with some oceanic influence. They may also spread into adjacent climatic conditions, growing in more exposed microtopes under submediterranean,climates. They are all epiphytic mosses that colonize the bark of trunks and branches of a number of different species of trees and shrubs in variable ecological contexts. They thrive especially in sclerophyllous forests, thickets with sparse trees and ornamental trees of urban parks, but also in deciduous forests at low-mountain or riversides. Occasionally, they can be also found on rocks in the same kind of environments.

Orthotrichum tenellum has the largest confirmed distribution and widest ecological range. It spans all the Mediterranean Basin from the Iberian Peninsula and the Maghreb to southern Turkey and Cyprus through southern Europe. It is one of the most characteristic species of the European epiphytic bryoflora (Draper et al., 2008; Lara et al., 2001) and is also present in Macaronesia (Azores, Madeira and Canary Islands). *Orthotrichum tenellum* can also be found in relatively dry and warm niches along the central and northern Europe, from the British Isles to Ukraine, and reaching eastwards central Asia (Ignatov et al., 2006).

The other Old-World species, *O. comosum*, is only known from the Western Mediterranean Basin and the Canary Islands (Fig. IV.5.13); namely, it is relatively frequent in the south and western Iberian Peninsula and northern Morocco. It has been also recorded in La Gomera as *O. alpestre* (González Mancebo et al., 2007) and in northern Sicily. Its ecological range is similar to that of *O. tenellum*, but it is less frequent and generally prefers dryer niches.

The four American species seem to be restricted to the state of California, although the presence of populations in adjacent states (especially the Mexican Baja California) should not be discarded. They all spread through the Coastal Ranges and the Pacific coast with Mediterranean climate, between Point Arena and Gulf of Santa Catalina, including the Channel Islands. In these coastal areas they are found coexisting and mixed in the same epiphytic communities of sclerophyllous forests and thickets. *Orthotrichum franciscanum* exhibits the most oceanic affinity, growing usually in lowlands, at 0–150(–390) m of altitude, rarely venturing more than 20 km inland. It is especially frequent in San Francisco Bay, even in urban parks and tree-lined streets, and it also colonizes granite boulders at the Farallon Islands (Fig. IV.5.13). The distribution of *O. coulteri* is broadly similar to the latter, although it is not so frequent in urban areas and is easier to be found at higher altitudes (20–600 m of altitude). The two remaining species are more widespread, reaching the Great Valley and the western slopes of Sierra Nevada. *Orthotrichum cucullatum* has been collected from the centre of Sacramento Valley to the north of the Peninsular Ranges (Fig. IV.5.13), through an altitude range of 15–600 m. Finally, *O. norrisii* shows a very similar distribution (Medina et al., 2008) reaching northwards the Klamath Mountains and ascending up to 900 m.

Our results revealed a complex pattern in the phylogeny of this wide concept of *O. tenellum* that indeed hosts six species, all of them morphologically diagnosable. Furthermore, among the species used for the phylogenetic reconstruction, very different biogeographic patterns can be found. There are endemics of the Californian Region that grow simpatrically, often sharing the same environments and in the same epiphytic communities: *Orthotrichum coulteri*, *O. cucullatum* and *O. franciscanum*. In turn, there are two sister species whose phylogenetic proximity suggests a very recent speciation event but are distributed in disjunct, distant areas: *Orthotrichum tenellum* and *O. norrisii*. Finally transcontinental disjunctions do also occur among other *Orthotrichum* species (*Orthotrichum columbicum* and *O. pulchellum*), although this has not been the case of the taxa included in the former concept of *O. tenellum*. We argue that the specialized morphological revision has been a fundamental work for this study, allowing us to target adequately the DNA sequencing with a solid hypothesis. This situation may not be just an exception in bryophyte biodiversity, and a critical reevaluation of morphological delimitations is advised prior to phylogenetic reconstructions or broad generalizations.

Key to species. In order to facilitate the recognition of studied taxa from those that could be confused with them in the Mediterranean Basin, Californian Region and adjacent areas, we provide an artificial key to *Orthotrichum* species with cryptopore stomata in the lower half of the urn. Some species that are not meaningfully subject of confusion are excluded of this key: taxa without developed exostomes (Garilleti et al., 2011); taxa with erect exostomes (subgenus *Orthotrichum* sensu Lewinsky, 1993); riparian species

(Lewinsky-Haapasaari & Norris, 1998a) and taxa with mostly bistratose leaf laminae (Norris et al., 2004) or margins (Lewinsky-Haapasaari & Norris, 1998b).

1. Capsules exserted..**2**

1'. Capsules immersed..**6**

2. Endostome of 16 segments (sometimes the 8 intermediary ones reduced), exostome PPL with conspicuous longitudinal striae, operculum with a wide bright red to crimson basal rim, seta commonly 2.5–5.5 mm long, calyptra always naked ....***O. columbicum***

2'. Endostome of 8 segments, exostome PPL smooth or almost smooth, operculum with thin orange basal rim, seta commonly 1.0–2.0 mm, calyptra naked or with more or less abundant hairs..**3**

3. Vaginula and calyptra clearly hairy, stomata always in the lower half of the urn and neck..**4**

3'. Vaginula naked or very sparsely hairy, calyptra naked or hairy, stomata mainly in the middle urn (rarely in the lower half, never on the neck)..**5**

4. Leaf margins recurved along most of the lamina, leaf apices acute or blunt, without differentiated hyaline cells, connective membrane low and inconspicuous ....***O. coulteri***

4'. At least one of the leaf margins plane or almost so, frequently both, leaf apices often ending in one or a few enlarged hyaline cells, connective membrane distinctly high..***O. persimile***

5. Calyptra with abundant to scarce papillose hairs, vaginula (0.25–)0.4–0.8 mm long, naked or with sparse papillose hairs, spores commonly 8–13 µm ....***O. consimile***

5'. Calyptra naked, exceptionally with very few inconspicuous and smooth hairs, vaginula very long (0.6–)0.8–1.1(–1.3) mm, naked, spores commonly 12–16 µm ....***O. confusum***

6. At least some leaves with channeled apices..**7**

6'. Leaves without channeled apices...**11**

7. Leaves widely oblong with apices abruptly cuspidate into a channel, rhizoids distinctly ascending along the stems...***O. bartramii***

7'. Leaves linear-lanceolate to ovate-lanceolate, more or less gradually tapering to a channeled apex, rhizoids not distinctly ascending along the stems
....**8**

8. Axillary hairs 700–1200 µm long, multiseriate and branched, in dense fascicles, forming a tomentum on the stems..***O. pilosissimum***

8'. Axillary hairs 75–250 µm long, filiform or subclaviform, uniseriate, not branched
....**9**

9. Exothecial bands 4–6 cell rows wide, capsules constricted below the mouth when dry and empty...***O. tenellum***

9'. Exothecial bands 2(–3) cell rows wide, capsules cylindrical, not or only slightly constricted below the mouth when dry and empty...**10**

10. Endostome of 16 segments (sometimes the intermediary reduced), vaginula mostly naked, operculum convex, orange, without a dark red, basal ring
....***O. scanicum***

10'. Endostome of 8 segments, vaginula mostly hairy, operculum plane or almost plane, with a dark red basal ring...***O. norrisii***

11. Leaf apices obtuse or rounded and frequently cucullate......................***O. cucullatum***

11'. Leaf apices various, mostly acute to acuminate, but never rounded and cucullate
....**12**

12. Leaf apices acute to acuminate, ending in one or a few elongated, hyaline cells, leaf margin revolute at mid-leaf, exostome teeth often truncated
....***O. franciscanum***

12'. Leaf apices various, but not ending in hyaline cells, leaf margins recurved, exostome teeth triangular, not truncated..**13**

13. Exothecial bands 2(–3) cell rows wide..**14**

13'. Exothecial bands 4–6 cell rows wide..**15**

14. Exostome pairs of teeth remaining fused when recurved, endostome of 8 segmens. ....***O. patens***

14'. Exostome pairs of teeth splitting when recurved, endostome of 16 segments ....***O. underwoodii***

15. Capsules with ribs marked only in the upper half, exostome pairs of teeth fused in pairs only at base and irregularly recurved when dry, endostome clearly papillose ....***O. handiense***

15'. Capsules with ribs well marked along almost the whole urn length, exostome pairs of teeth remaining intact and regularly recurved when dry, endostome smooth or slightly ornamented..**16**

16. Exostome teeth with dense papillae in the lower part and striate-papillose towards apex, vaginula naked or hairy, calyptra with abundant, thick-walled hairs 17

16'. Exostome teeth uniformly papillose, vaginula hairy, calyptra naked or with thin and scarcely papillose hairs..**18**

17. Mid-leaf cells smooth or with low papillae, vaginula naked, leaf surface often with gemmae, calyptra hairs typically forming an apical comma ....***O. comosum***

17'. Mid-leaf cells with high and branched papillae, vaginula hairy, leaf surface rarely producing gemmae, calyptra hairs evenly distributed, not forming a comma ....***O. alpestre***

18. Vaginula with long and papillose hairs, calyptra hairy, capsule neck long defluent ....***O. stramineum***

18'. Vaginula usually with abundant, short and smooth hairs, calyptra naked, capsule neck shortly defluent..***O. stellatum***

Species description

Orthotrichum tenellum Bruch ex Brid., Bryol. Univ. 1: 786. (1827). Type: "prope Bipontium" *Bruch* (Lectotype: B!)

Figs. IV.5.3 and IV.5.4

Plants 0.3–1.2(–1.6) cm tall in dense tufts, light green to blackish. *Rhizoids* reddish-brown, smooth or somewhat rough, at the lower parts. *Stems* branched, subpentagonal in section with 1–3 layered sclerodermis of thick-walled cells. *Axillary hairs* 50–100(–125) µm, filiform or subclaviform, 3–5 cells long, the 1–2 basalmost brown and shorter. *Leaves* (1.7–)2.0–2.8(–3.2) x (0.3–)0.5–1.0 mm, ligulate to ovate-ligulate, sometimes ovate-lanceolate, appressed when dry, erect-patent when moist; leaf apex rounded to subacute, rarely acute, very often with incurvate margins forming a distinct channel; leaf margins entire, recurved along most of the leaf length; leaf lamina unistratose, somewhat keeled; costa 40–70 µm wide at base, 30–45 µm at mid-leaf, with two rows of ventral cells, ending below leaf apex. Basal leaf cells (20–)35–60(–75) x (8–)15–22 µm rectangular to elongate, thin or thick-walled; marginal basal cells 9–15 x 6–12 µm rectangular to subquadrate; mid-leaf cells 9–16(–20) x 8–15 µm isodiametric to elongate, smooth or with 2–3 low papillae. *Gemmae* 150–200(–350) µm, 4-7 cells long, green to brownish, fusiform or claviform, frequent and often abundant on leaves.

Cladautoicous; perigonia terminal on male branches, perigonial leaves ovate to elliptical with short costa and rhomboidal cells; perichaetial leaves terminal on female branches, perichaetial leaves not differentiated. *Vaginula* 0.3–0.6 mm long, cylindrical to frustrum-shaped, naked. *Calyptra* fusiform when young, fusiform to oblong-conic when mature, longitudinally plicate, with sparse (uni)biseriate, appressed, smooth hairs up to 0.8(–1.1) mm long, rarely densely growing, forming a terminal comma. *Seta* 0.4–0.8(–1.0) mm, not twisted. *Capsule* immersed to shortly emergent, 1.2–2.5 mm in length, fusiform when full of spores, subcylindrical, constricted below mouth and strongly ribbed when dry and empty; capsule neck defluent. Lid subconic or somewhat convex, rarely plane, with short rostrum and a reddish or orange thin basal ring. Exothecial cells 20–45 x 8–20 µm, hyaline, elongated; 8 exothecial bands differentiated along most of the urn length, (3–)4–6 cells wide, band cells (12–)30–50 x 12–25(–30) µm, rectangular, thick-walled and brown. *Stomata* cryptopore partially to completely covered by surrounding cells, located in the lower half of the urn, very rarely reaching the neck. *Peristome* double. *Exostome* of 8 pairs of teeth 150–200(–250) µm long, triangular, whitish to pale brown, very rarely splitting, recurved when dry; external side of the teeth (exostome Outer Peristome Layer, OPL) densely papillose in all its length, very rarely with isolated striae towards apex; internal side of the teeth (exostome Principal Peristome Layer, PPL) smooth, sometimes with low papillae and striae towards apex. *Endostome* of 8 segments, 140–190(–220) µm

FIG. IV.5.3. *Orthotrichum tenellum*. A: habit; B: calyptra with sparse hairs; C-E: different stages of capsule development; F: naked vaginula, seta and lower half of the capsule; G: leaf variability; H,I: leaf apices with incurved margins, forming a distinct channel. A-F: Greece, Rendina Lara et al. 1994; G,H: Spain, Viso del Marqués *Medina & Medina 2006*; I: Spain, Aracena *Albertos et al. 1998* (Herb. at Univ. Autónoma de Madrid and VAL).

long, linear or somewhat stout; external side of the segments (endostome PPL) smooth; internal side of the segments (Inner Peristome Layer, IPL) more or less papillose or striate, very rarely smooth; connective membrane low and often fragmented, ornamented like the endostome. *Spores* 12–17(–21) µm, subspheric, papillose.

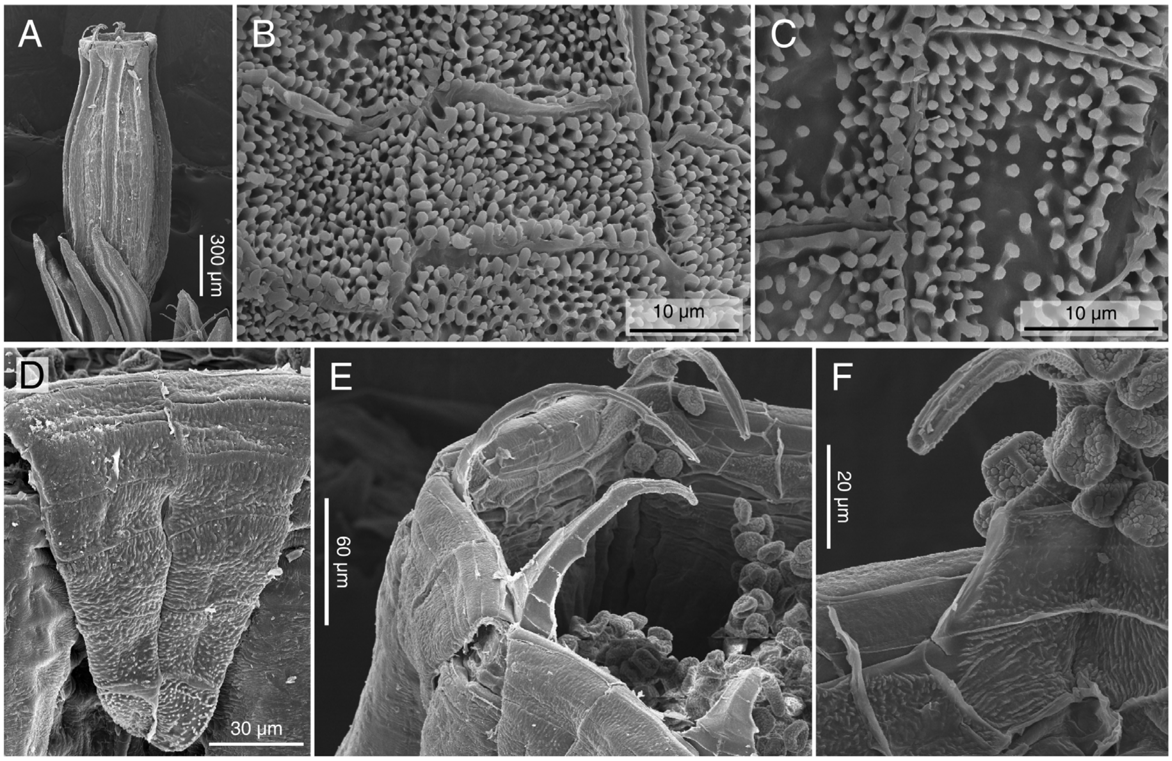


FIG. IV.5.4. *Orthotrichum tenellum*. A: capsule; B: exostome basal OPL showing dense papillae; C: exostome apical OPL with papillose ornamentation; D: exostome PPL with low papillae and striae towards teeth apices; E: detail of the peristome showing the smooth endostome PPL; F: endostome IPL and basal membrane ornamented with low papillae and striae. A-F: Greece, Rendina *Lara et al*. 1994 (Herb. at Univ. Autónoma de Madrid and VAL).

***Orthotrichum comosum sp. nov*. F. Lara, R. Medina & Garilleti**

> *Plantae humiles, usque ad 0.6 cm altae, confertim frondosae. Folia linearia vel ovato-lanceolata, marginibus acutis, acuminatis sive in mucrone cellularum viridium, non canaliculatis, terminantia, lamina pauce papillosa, marginibus recurvatis.Vaginula nuda, sed calyptra ad apicem proprie comosa. Capsula immersa striataque, stomatibus cryptopora ex dimidio usque ad collum munita. Peristoma duplex. Exostoma octo dentium paribus recurvatis et striatis ad apicem, endostoma octo segmentis.*

HOLOYPE: SPAIN. Cádiz, Algeciras, Sierra de la Luna, *Quercus* forest close to los Llanos del Juncal, 36º06'02"N 05º32'14"W, 700m, epiphyte on *Quercus suber*; 13 March 1998 *J. Guerra, F. Lara & V. Mazimpaka* (VAL). Isotype at Universidad Autónoma de Madrid

Figs. IV.5.5 and IV.5.6

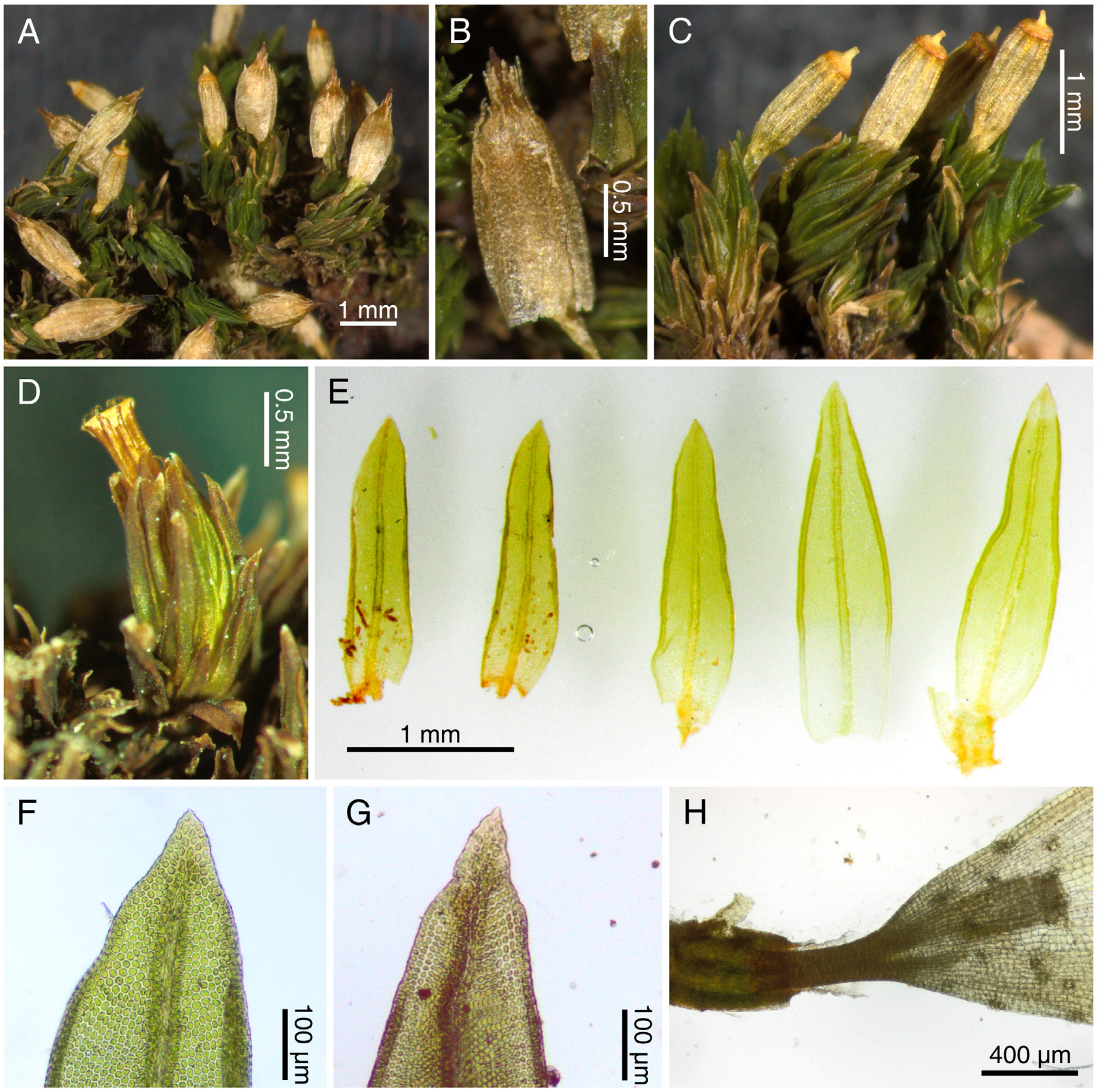


FIG. IV.5.5. Orthotrichum comosum. A: habit; B: calyptra with distinct apical comma of hairs; C,D: different stages of capsule development; E: leaf variability; F,G: leaf apices, acute or mucronate; H: naked vaginula, seta and lower capsule. A-C,E,F,H: holotype; D,G: Portugal, Morais, *Garilleti et al*. 2000 (Herb. at Univ. Autónoma de Madrid and VAL).

Plants up to 0.9 cm tall, in dense and small tufts, dark green. *Rhizoids* reddish–brown, smooth or slighly rugose, on stem basal parts. *Stems* branched, densely foliose, rounded to subpentagonal in section, sclerodermis of 2–3 thick-walled cell layers. *Axillary hairs* 50–125(–200) µm long, subclaviform to filiform, 3–5 cells, the basalmost shorter and brown. *Leaves* 1.75–3.0 x 0.5–0.8 mm, linear-lanceolate to ovate-lanceolate, with the base sometimes widened, appressed when dry, erect-patent when moist; leaf apex plane or slighltly keeled, blunt-acute to acuminate, often mucronate; leaf margins recurved to revolute from base to upper third or almost to apex; lamina unistratose, occasionally with bistratose ridges scattered towards the upper part, keeled in section; costa 65–80(–100) µm wide at base, 47–60 µm at mid-leaf, with two guide cells, ending below apex. Basal leaf cells (25–)35–85 x 10–19 µm, subrectangular or elongate to somewhat elliptical, with walls thin or thickened, sometimes nodulose; marginal basal cells 17–33 x 13–21

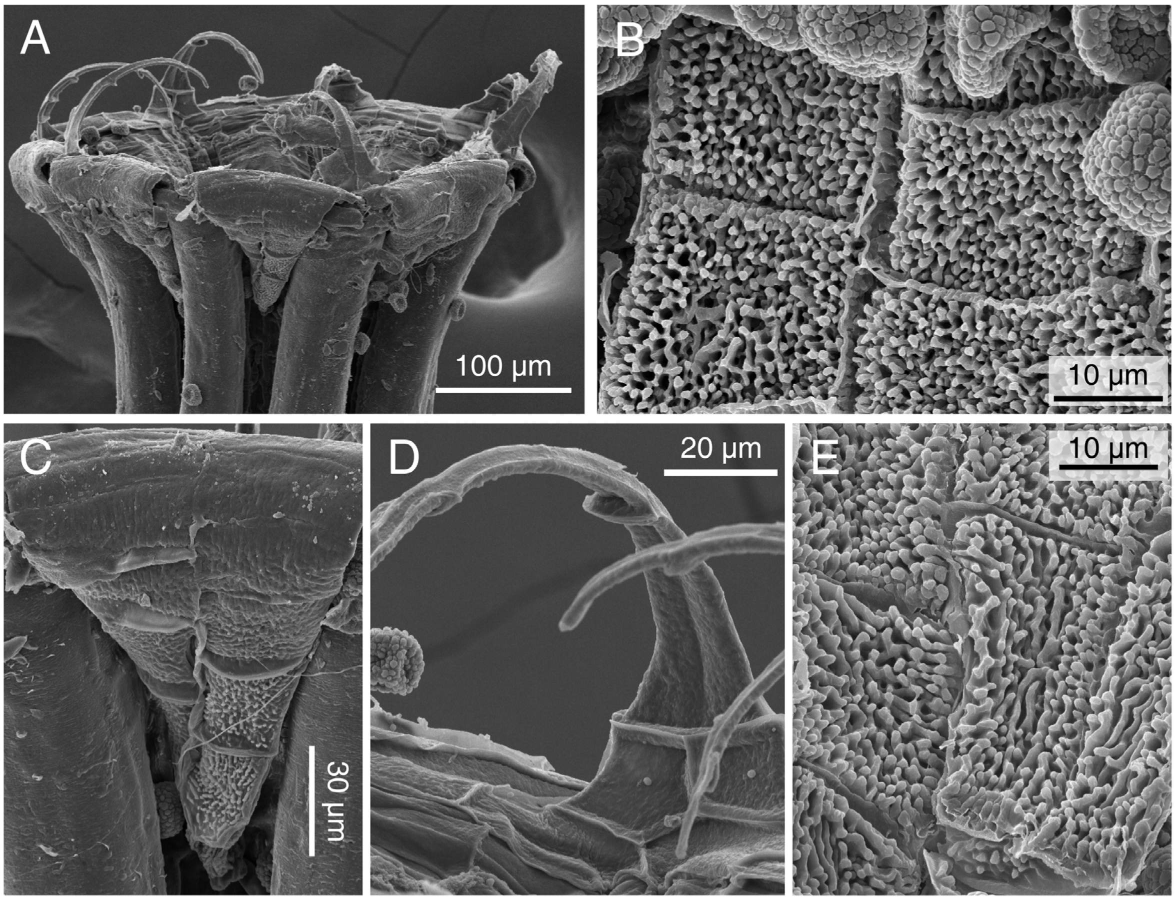


FIG. IV.5.6. *Orthotrichum comosum*. A: peristome and upper capsule; B: exostome basal OPL, densely papillose; C: exostome pair of teeth internal view (PPL), with low papillae and striae towards apex; D: view of an endostome segment with almost smooth surfaces; E: apical exostome OPL, with papillae and lines. A: Portugal, Morais, *Garilleti et al. 2000*; B-E: holotype (Herb. at Univ. Autónoma de Madrid and VAL).

µm, subquadrate to rectangular; middle and upper cells (7)11–21 x 9–15 µm, isodiametric or elongated, sometimes irregular, smooth or with (1–)2(–3) low unbranched papillae. *Gemmae* 75–300(–500) x 22–30 µm, vermiform or claviform, very rarely branched, 4–20 cells long, frequent on leaves.

Goniautoucous or cladoautoicous, perigonia lateral or terminal, frequently adjacent to perichaetia, perigonial leaves oblong-elliptical, apiculate with rhomboidal or polygonal cells; perichaetia terminal, perichaetial leaves not differentiated, ocasionally elliptical-ligulate with plane margins. *Vaginula* 0.25–0.4(–0.6) mm, cylindrical or frustum-shaped, naked. *Calyptra* fusiform when immature, turning oblong-conic and longitudinally plicated when mature, typically with an apical comma of robust, uni-biseriate hairs up to 600 µm long, papillose and thick-walled. *Seta* 0.3–0.6 mm, not twisted. *Capsule* immersed,1.3–1.8(–2.0) mm long, fusiform to subcylindrical when full of spores, turning cylindrical, strongly ribbed and contracted below the mouth when empty and dry; capsule neck very defluent; Lid plane or slightly convex, with a short rostrum and a light orange basal ring. *Exothecial cells* (15–)25–55(–70) x 11–24(–28) µm, elongated, subrectangular or polygonal, hyaline; 8 exothecial bands reaching the lower third of the urn, 4–5 cells wide, band cells (18–)35–55(–67) x 14–33(–41) µm, thick-walled and brownish. *Stomata* cryptopore, partially to completely covered by surrounding cells, present in the lowerhalf (third) of the urn and the neck. *Peristome* double. *Exostome* of 8 pairs of teeth, 180–250 µm long, very rarely splitting when old, triangular, whitish to brownish; OPL with a more or less papillose basal reticule, sometimes with smooth areas, papillose and with vermicular lines towards apex; PPL smooth at base, smooth or with faintpapillae and longitudinal striae towards apex. *Endostome* of 8 segments 125–230 µm long, with broad bases and usually thickened transversal walls; PPL smooth; IPL rugulose, sometimes papillose, rarely with striae at bases; connective membrane continuous, low, ornamented like segment bases. *Spores* (10–)12–16(–18) µm, subspheric, coarsely papillose.

Etymology

The specific epithet refers to the distinct hairy comma of the calyptra.

Orthotrichum coulteri Mitt., J. Linn. Soc. Bot. 8:25. (1864). Type: California *Coulter 839-840* (Lectotype: FH!)

Orthotrichum cylindrocarpum Lesq., Trans. Am. Phil. Soc. 13: 6. (1865). Type: California, Oakland, *Bolander* (Lectotype MICH!, Isotypes CANM, FH!, NY!)

Orthotrichum tenellum var. coulteri (Mitt.) Grout, N. Am. Fl. 15A:19 (1946)

Orthotrichum tenellum var. cylindrocarpum (Lesq.) Koch, Leafl. West. Bot. 6: 23. (1950)

Figs IV.5.7 and IV.5.8

Plants 0.3–0.8(–1.4) cm tall, forming loose tufts, light to dark green. *Rhizoids* brown-reddish, smooth or slightly rough, on stems lower parts and very frequently on branches, sometimes bearing gemmae. *Stems* densely branched, subpentagonal in section with a 1–2(–3) layered sclerodermis of thick-walled cells. *Axillary hairs* 100–150(–225) µm, filiform, comprised of 3–4(–7) cells, the basalmost brown and shorter. *Leaves* (1.2–)1.5–2.7(–3.2) x 0.2–0,5(–0.7) mm, long triangular to narrowly lanceolate, rarely ovate-lanceolate, appressed when dry, erect-patent when moist; leaf apex acute to subobtuse, blunt, not channelled; leaf margins entire, recurved from base to near apex; leaf lamina unistratose, rarely with scattered bistratose ridges towards the upper part, keeled in section; costa 75–90 µm wide at base, 45–60µm at middle lamina, with two rows of ventral cells, ending below apex. Basal leaf cells (12–)20–50(–62) x (5–)7.5–15 µm, hyaline, rectangular to polygonal, generally thick-walled; marginal basal cells (5–)10–17 x

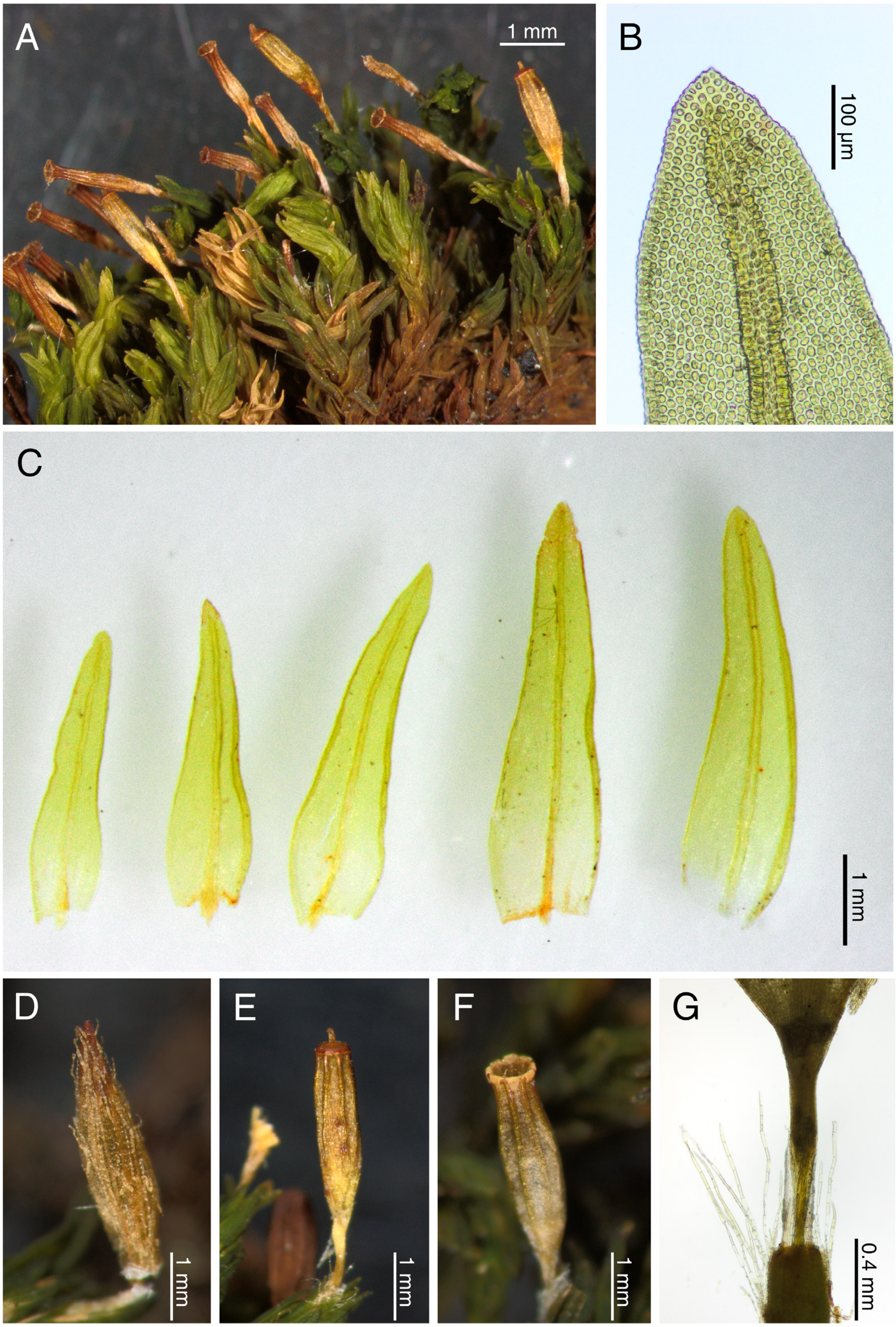


FIG. IV.5.7. *Orthotrichum coulteri*. A: habit; B: blunt leaf apex; C: leaf variability; D: densely hairy calyptra; E,F: different stages of capsule development; G: vaginula, bearing abundant, long hairs, and seta. A-C,E: California, Wildcat Canyon *Lara et al. 20.IX.2008*; D,F,G: California, Berkeley, *Lara & Garilleti 18.IX.2008* (Herb. at Univ. Autónoma de Madrid and VAL).

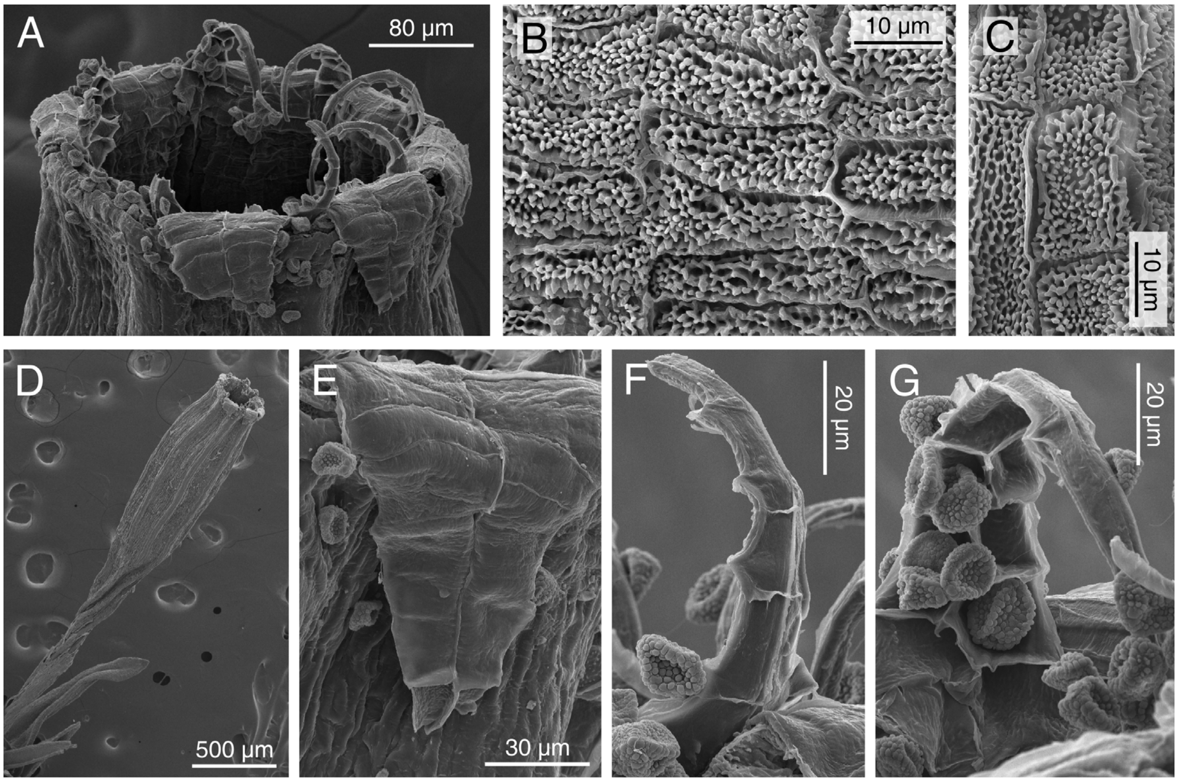


FIG. IV.5.8. *Orthotrichum coulteri*. A: peristome. B: exostome basal OPL, with a densely papillose ornamentation; C: papillose surface, with scattered lines, of the exostome apical OPL; D: seta and capsule; E: smooth surface of the exostome PPL; F: endostome segment showing the smooth PPL; G: slightly rough internal side of an endostome (IPL). A-G: California, Wildcat Canyon *Lara et al. 20.IX.2008* (Herb. at Univ. Autónoma de Madrid and VAL).

10–20 µm, subquadrate; middle and upper cells 7.5–15(–18) x (5–)10–12 µm, isodiametric to elongate with (1–)2–3(–4) low papillae, rarely smooth. *Gemmae* 45–125(–250) x 20–30 µm, fusiform to claviform, occasionally long and branched, formed by 3–9 cells, frequent and sometimes very abundant on leaves, rare on rhizoids.

Cladoautoicous; perigonia terminal on male branches; perigonial leaves ovate with short costa and rhomboidal cells; perichaetia terminal on female branches, perichaetial leaves not differentiated. *Vaginula* (0.5–)0.6–0.8 mm long, cylindrical to frustum-shaped, with abundant uni-biseriate hairs, (0.2–)0.4–0.9(–1.5) mm, with thick-walled cells, often papillose. *Calyptra* conic when young, narrowly oblong-conic or fusiform when mature, yellowish and longitudinally ribbed, with abundant, conspicuous (uni-biseriate hairs, papillose and thick-walled. *Seta* 0.8–1.5(–2.2) mm, yellowish, twisted counterclockwise. *Capsule* exserted, rarely almost so, (1.3–)1.5–1.75(–2.1) mm in length, fusiform when full of spores, turning cylindrical, longitudinally ribbed and somewhat constricted below mouth when empty and dry; capsule neck defluent. *Lid* plane to subconic, rostrate, with a darker reddish basal ring. Exothecial cells 15–45(–60) x (10–)12–15 µm, subrectangular to elongate, hyaline; 8 exothecial bands differentiated along most of the urn, (2–)3–4 cells wide, band cells (12–)20–40(–52) x (12–)20–27 µm, subquadrate to rectangular, brown and thick–walled. *Stomata* cryptopore almost to completely covered by surrounding cells, restricted to the lower third of the urn and the neck. Peristome double. *Exostome* of 8 triangular pairs of teeth, 170–210 µm long, not splitting when old, pale yelowish, recurvedwhen dry; (OPL) uniformly papillose-reticulate, rarely forming vermicular lines towards apex; (PPL) smooth, or rarely with very subtle striae. *Endostome* of 8 segments narrowly triangular to linear, alternating with exostome teeth,125–150(–175) µm, (PPL) smooth or with subtle basal striate and apical papillae; (IPL) smooth; connective membrane low, ocasionally discontinuous, ornamented like the endostome. *Spores* 12–18(–22) µm, subspheric, papillose.

***Orthotrichum cucullatum sp. nov*. F. Lara, R. Medina & Garilleti**

> *Plantae ad 0.8 cm altae. Folia ligulata vel ovato-ligulata, marginibus recurvatis, apicibus obtusis rotundatisve, persaepe proprie cucullatis, non canaliculatis. Vaginula generatim pilosa, sed calyptra nuda vel parce pilifera, ovoidea rostrataque. Capsula immersa, striataque, stomatibus cryptoporibus in dimidio inferiore, collo excluso. Peristoma duplex. Exostoma octo dentium paribus recurvatis, endostoma octo segmentis.*

HOLOTYPE: U.S.A. California. Alameda Co., Berkeley, Tilden Regional Park. Botanical Garden, on trunk of *Aesculus californica*, 18 October 2008 *Lara* (CAS). Isotypes at Universidad Autónoma de Madrid and VAL.

Figs: IV.5.9 and IV.5.10

Plants 0.3–0.8(–1.2) cm tall, in compact tufts, light to bright green. *Rhizoids* reddish-brown, on lower parts of the stems, smooth or slightly rough. *Stems* branched below, subpentagonal in section with a 1–3 layered sclerodermis of thick-walled cells. *Axillary hairs* (75–)100–175(–200) µm long, filiform to subclaviform, comprised of (3–)4–7 cells, the 1-2 basalmost shorter and brown. *Leaves* (1.2–)1.5–2.5(–2.75) x (0.3–)0.4–0.6(–0.75) mm, ligulate to ovate-ligulate, appressed, straight or occasionally weakly flexuose when dry, erect-patent when moist; leaf apex rounded to obtuse, almost plane to cuculate, sometimes denticulate; leaf margins entire, recurved through most of the lamina; lamina unistratose, variably keeled in section; costa 55–75 µm wide at base, 40–50 µm at mid-leaf, with two rows of ventral guide cells, ending below apex. Basal leaf cells (25–)35–

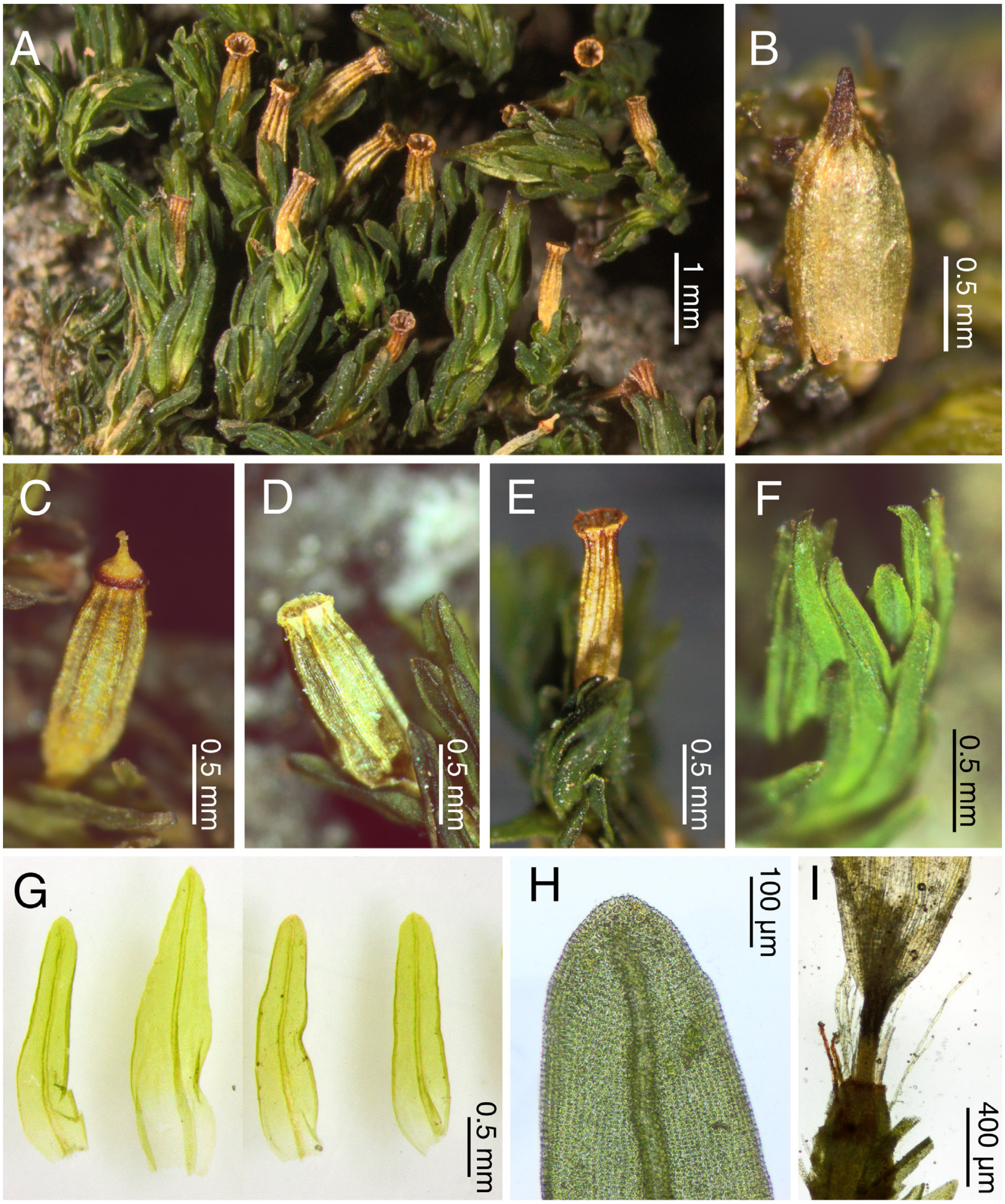


FIG. IV.5.9. *Orthotrichum cucullatum*. A: habit; B: calyptra with sparse, short hairs; C-E: different stages of capsule development; F: leaves with distinct cucullate apices; G: leaf variability; H: rounded leaf apex; I: hairy vaginula, seta and lower capsule. A,E-I: holotype; B-D:California, Aromas *Kellman 929* (CAS-1017506);

60(–80) x 12–18(–24) µm, rectangular to elongate, hyaline, mostly thin-walled; marginal basal cells 10–14 x 12–16 µm, subquadrate; middle and upper leaf cells (7–)10–14(–20) x 7–12 µm, isodiametric to elliptical, with 2-3 papillae. *Gemmae* 50–80 µm, fusiform to claviform 3–5 cells long, usually present on leaves, but scarce.

Cladoautoicous; perigonia terminal at male branches; perigonial leaves ovate to ovate-lanceolate with short costa and rhomboidal cells; perichaetia terminal at female branches, perichaetial leaves not differenciated. *Vaginula* 0.2–0.3(–0.5) mm long, cylindrical or

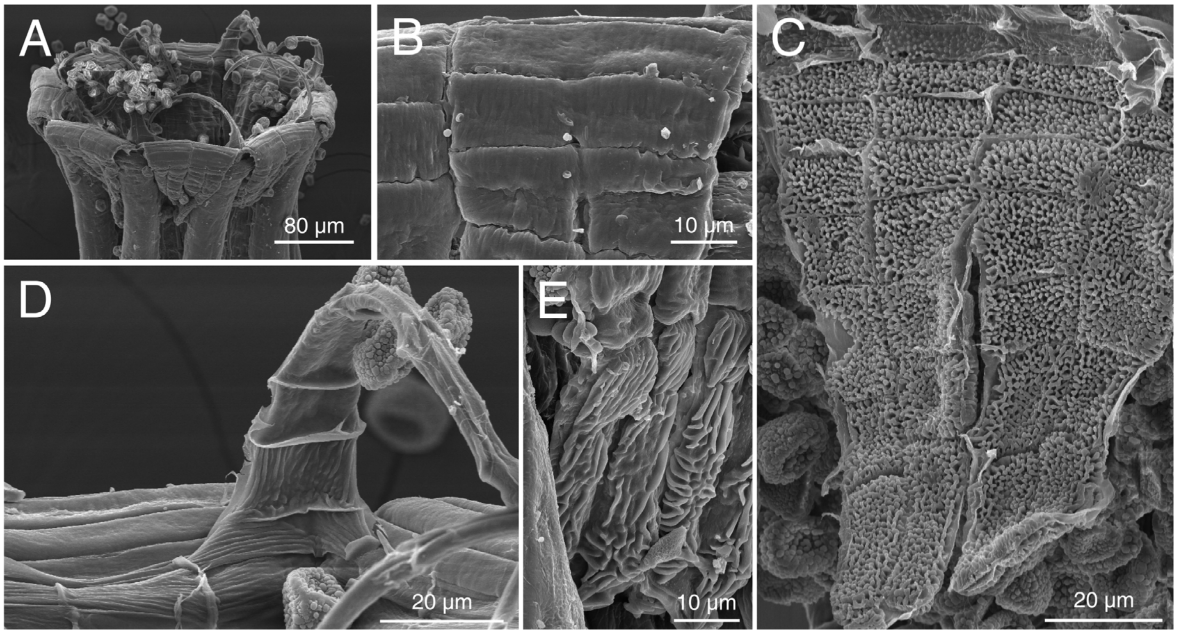


FIG. IV.5.10. *Orthotrichum cucullatum*. A: peristome; B: smooth surface of the basal exostome PPL; C: densely papillose exostome OPL; D: detail of an endostome segment, showing striae at the basal IPL; E: apical exostome PPL with striate ornamentation. A,B,D,E: holotype; C: California, Corralitos *Kellman 719* (CAS-718768).

frustum-shaped, with scarce uni-biseriate hairs (150–)200–500(–600) µm, exceptionally naked. *Calyptra* ovoid-conic when young, turning ovoid and rostrate to oblong-conic when mature, naked or with unconspicuous stout hairs up to 300 µm long, not concentrated at tip, longitudinally plicate. *Seta* (0.35–)0,5–0,75 mm long, twisted counterclockwise. *Capsule* immersed, (1.25–)1.5–1.75 mm long, cylindrical to fusiform and slightly constricted under mouth when full of spores, becoming subcylindrical, ribbed, gradually constricted from mouth to lower third (half) of urn when empty and dry; capsule neck defluent. *Lid* subconic with thick rostrum and dark reddish basal ring. *Exothecial* cells (20–)30–50 x (10–)12–16 µm shortly rectangular to elongate, hyaline; 8 exothecial bands differentiated through most of the urn length, 4(–6) cells rows wide, band cells 25–55 x (12–)16–30 µm, thick-walled and brownish. *Stomata* cryptopore, almost to completely covered by surrounding cells, present at lower half of capsule but not on neck. *Peristome* double. *Exostome* of 8 pairs of teeth, 160–210 µm long, long triangular, yellowish to orange, sometimes truncate and splitting into pairs when old, reflexed when mature and dry; OPL ornamented with a dense reticulum more or less papillose, sometimes forming vermicular lines towards apex, but often eroded; PPL smooth at base, often striate towards apex. *Endostome* of 8 segments alternating with teeth, 110–180 µm long, linear, somewhat widened at base, exceptionally with rudiments of intermediary segments; PPL smooth; IPL smooth or weakly ornamented; connective membrane low, continuous or fragmented. *Spores* 12–16(–20) µm, subspheric, papillose.

Etymology

The specific epithet mentions the cucullate leaf apices.

***Orthotrichum franciscanum* sp. nov.**

> *Plantae humiles, ad 0.8 cm altae. Folia linearia, lanceolata vel ovato-lanceolata, apicibus planis, acutis acuminatisve, persaepe in elongatas cellulas hyalinas terminantia; marginibus recurvatis, sed ad laminae dimidio revolutis. Vaginula calyptraque variabile piliferae. Capsula immersa (emergens), 8-striata, cryptoporibus stomatibus in dimidio inferiore usque ad collum. Peristoma duplex. Exostoma octo dentium paribus recurvatis, saepe ad apices fenestratis, endostoma octo segmentis.*

HOLOTYPE: U.S.A. California. San Francisco Co., Holly Park, off Holly Drive, 37º44'15"N 122º25'10"W, 300 ft; on trunk of *Eucalyptus*, near top of the hill, 1 April 2000, *Shevock 19132* (UC- 17419664). Isotype at Universidad Autónoma de Madrid.

Figs: IV.5.11 and IV.5.12

Plants (0.2–)0.4–0.8 cm tall, in small, loose tufts, olive green to blackish green. *Rhizoids* reddish-brown, smooth or rough, mostly restricted to stem lower parts. *Stems* densely branched, subpentagonal in section, with sclerodermis of 1–2(–3) thick-walled cell layers. *Axillary hairs* 75–200 µm long, filiform to subclaviform, comprised of 3–5 cells, the 1–2 basalmost shorter and brown. *Leaves* 1.2–2.5(–2.8) x 0.3–0.7(–0.9) mm, very variable in shape, from linear-lanceolate to ovate-lanceolate or ovate-ligulate, appressed when dry, erect-patent when moist; leaf apex plane, acute, frequently acuminate and ending in a fragile, linear apiculum of 1–3(–5) hyaline cells, (20–)27–55(–70) x 10–15 µm elongated to bullet-shaped; leaf margins entire, typically revolute at mid-leaf and only slighlty recurved towards the base and apex; lamina unistratose, keeled in section; costa (55–)70–90(–110) µm wide at base, (35–)45–60 µm at the mid-leaf, with 2 rows of ventral cells, ending below apex. Basal leaf cells (14–)20–48(–80) x (8–)10–22(–32) µm, elongate to polygonal, thin or thick-walled, locally sinuous; marginal basal cells (10–)15–37(–65) x (8–)12–15(–18) µm, rectangular to subquadrate, thick-walled; middle and upper leaf cells (8–)10–15(–22) x (8–)10–18(–20) µm, isodiametric to elliptical, sometimes subhexagonal or irregular, smooth or with 2–4 not prominent papillae; *Gemmae* 60–110(160) µm long, fusiform to cylindrical, 3–7 cells long, occasional on leaves.

Cladautoicous; perigonia on male branches, perigonial leaves ovate to ovate-lanceolate, with rhomboidal cells and short costa; perichaetia terminal on female branches, perichaetial leaves not differenciated. *Vaginula* 0.3–0.5 µm long, cylindrical to frustum-shaped, with sparse uni-biseriate hairs, (200–)400–700(–900) µm, frequently thick-walled and papillose. *Calyptra* fusiform when young, turning oblong–conic when mature, yellowish with brown or blackish tip, longitudinally plicate, with sparse uni-

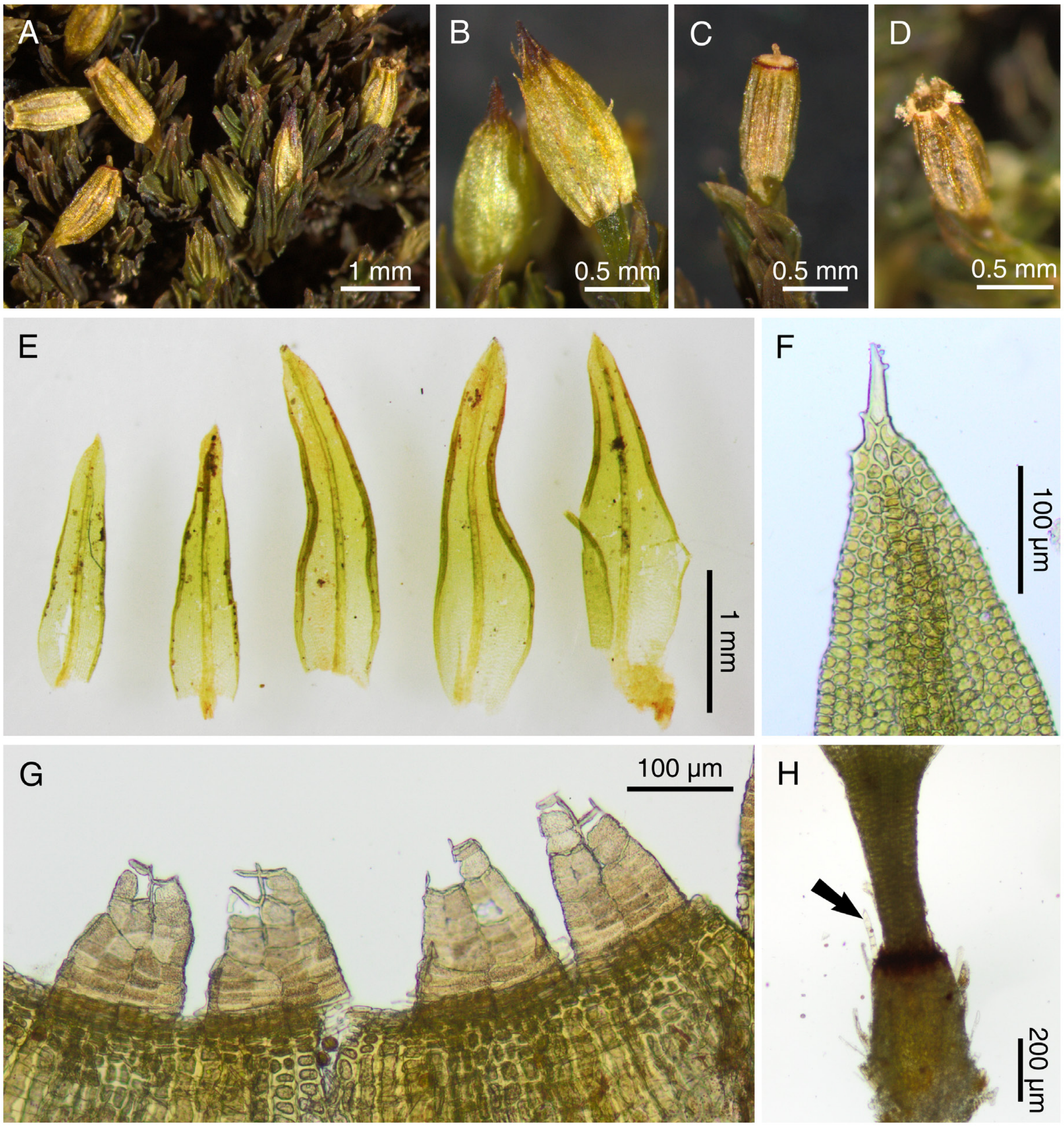


FIG. IV.5.11. *Orthotrichum franciscanum*. A: habit; B: calyptra with sparse, short hairs; C,D: different stages of capsule development; E: leaf variability, note revolute margins at mid-leaf; F: leaf apex with terminal, hyaline, elongate cell; G: distinct exostome teeth truncated at apex; H: almost naked vaginula. A,C: holotype; B,D-H: California, San Francisco *Shevock 20202* (UC-1741780).

biseriate, papillose and thick-walled hairs. Seta 0.4–0.8(–1.0) mm long, somewhat twisted counterclockwise; *Capsule* immersed to emergent, 1.0–1.7 mm long, ovoid-elliptical when full of spores, turning subcylindrical, strongly ribbed and variably constricted in the upper half when empty and dry; neck very defluent; Lid plane or almost plane, with a thin and short rostrum and a red-brownish basal ring. *Exothecial* cells 22–50(–70) x (8–)12–17(–27) µm, rectangular or elongate, hyaline; 8 exothecial bands differentiated through most of the urn length, 3–4(–5) cells rows wide; band cells (16–)20–50 x (10–)15–28(–32) µm, rectangular, thick-walled and brownish. *Stomata* cryptopore, almost to completely covered by surrounding cells, restricted to the lower third (half) of the urn and neck. Peristome double; *Exostome* of 8 pairs of teeth, (130–)150–200(–240) µm

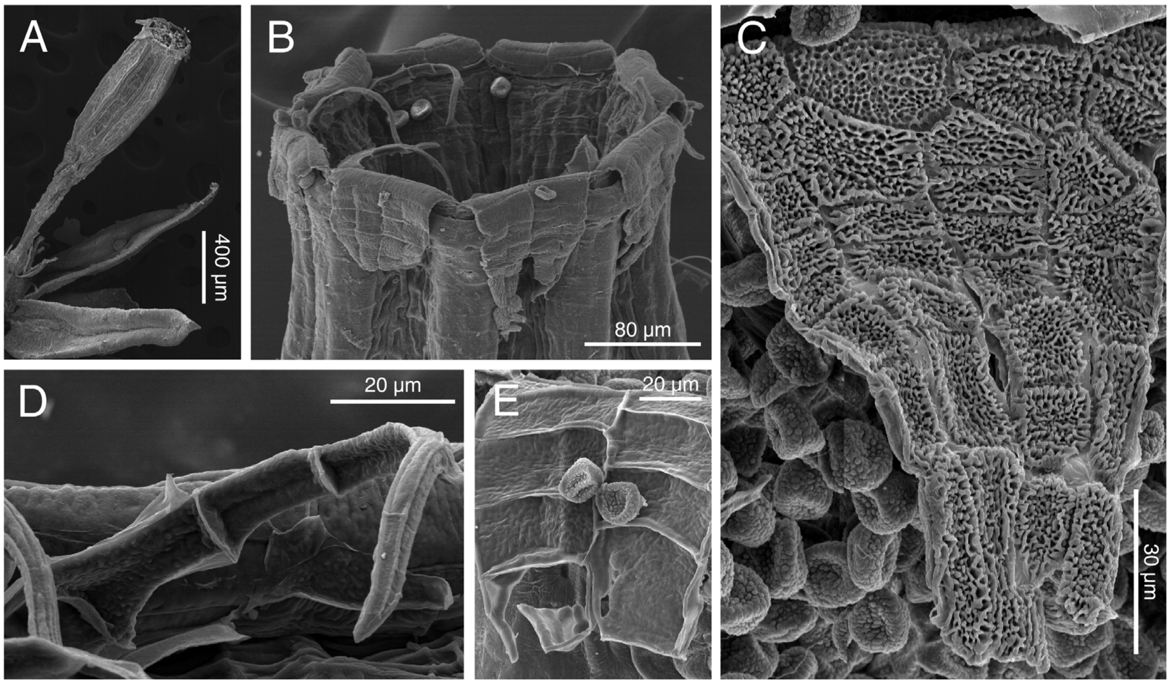


FIG. IV.5.12. *Orthotrichum franciscanum*. A: capsule; B: peristome; C: external surface of an exostome pair of teeth (OPL) showing papillae and striae; D: detail of an endostome segment with a very slightly ornamented IPL; E: exostome PPL surface of a truncated exostome tooth. A,B,D: California, Monterey 23.IX.2007; C: holotype; E: San Francisco *Shevock 20202* (UC-1741780).

long, triangular, whitish, non–splitting, recurved when dry, often truncated or fenestrated towards apex; OPL reticulate to papillose, sometimes forming faint transversal lines at the base, and longitudinal striae towards apex; PPL smooth or almost so. *Endostome* of 8 segments, (120–)140–170(200) µm long, linear with a wide base, frequently apendiculate; endostomial PPL smooth; IPL smooth or faintly papillose; connective membrane low and often fragmented, ornamented like the endostome. *Spores* (10–)12–16(–20) µm, subspheric, papillose.

Etymology

The specific epithet makes refers the frequence of this taxon in the San Francisco Bay area.

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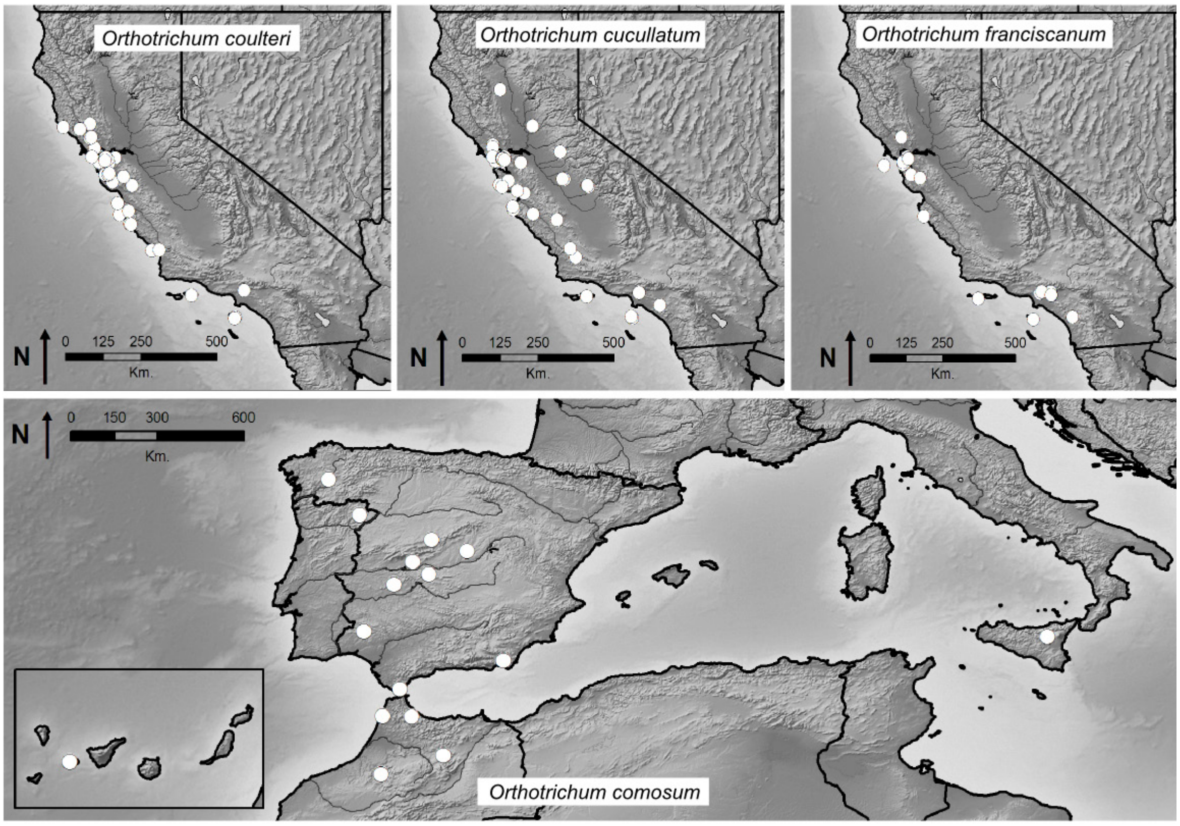

FIG. IV.5.13. Distribution of the selection of specimens of *Orthotrichum coulteri*, *O. cucullatum, O. franciscanum* and *O. comosum*.

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Appendix

Selection of specimens including voucher information (according to the guidelines, GenBank accession numbers will be provided after the manuscript is accepted). Vouchers without herbarium acronyms are kept at authors' herbarium Universidad Autónoma de Madrid. Specimens shown in Figs. IV.5.1 and IV.5.2 are preceded by a bracketed number.

Orthotrichum tenellum **CYPRUS. Nicosia:** Palaichori und Agros Mandelhain, 34,82826 33,11166,19. II.2009 *Frahm 2009744*; **GREECE. Ipiros:** Kalpaki, 30.VII.1999 *F. Lara, V. Mazimpaka & M. J. Cano;* **Kriti:** Lasithi, Oropediou Lasithiou, 35º09’47”N 25º26’42”E, 14.VIII.2005 *R. Medina*; **Makedonia:** Rendina 4.VIII.1999 *Lara, Mazimpaka & Cano*; **ITALY. Sicily: [1]** Montesalici, 37º43’45”N 14º38’59”E, 25.VII.1998, *F. Lara et al*.; **[2]** Madonie, Piano Zucchi, 37º54’19”N 14º00’02”E, 27.VII.1998 *F. Lara et al*.; **PORTUGAL. Tras-os Montes e Alto Douro: [3]** Bragança, Gimonde, 23.XI.2000, *C. Garcia et al*.; **[4]** Porrais, 22.XI.2000, *C. Garcia et al*.; **SPAIN. Albacete:** Sierra del Calar del Mundo, Riópar, 38º29’17”N 2º20’53”W, 1.XI.1993, *F. Lara, R. Garilleti & B. Albertos*; **Ávila:** Tormellas, UTM: 30TTK8666, 9.XI.2003, *R. Medina*; Navalperal de Tormes, UTM: 30TUK0168, 10.XI.2003, *V. Mazimpaka et al*.; **Ciudad Real:** Viso del Marqués, 38º26’36”N 03º36’18”W, 14.VI.2006, *N. G. Medina & R. Medina*; **Granada:** Sierra Nevada, Bubión, 9.II.1996, *F. Lara et al*.; **Huelva:** Aracena, UTM: 29SQB1391, 12.II.1998, *B. Albertos et al*.; **Jaén:** Santiago Pontones, UTM: 30SWH43, 23.I.1998, *F. Lara*; **Madrid: [7]** Cadalso de los Vidrios, 14.V.2006, *B. Estébanez & N. G. Medina* ; **Sta Cruz de Tenerife:** El Hierro, road Valverde-Frontera, UTM: 28RBR0673, 29.IX.2002, *F. Lara & E. San Miguel*; **[5]** La Palma, Barlovento, 4.XI.1997, *F.Lara*; **TUNISIA. Béja:** road Sejenane-Nefza, 37º02’40”N 9º06’29”E, III.2005, *F. Lara & E. San Miguel*; **Jendouba: [6]** near Fenana, 36º42’05”N 8º40’36”E, 22.III.2005, *F. Lara & E. San Miguel*;

Orthotrichum comosum. **ITALY. Enna:** Monte Salici, 37º43’45”N 14º38’59”E, 25.VII.1998, *Lara et al*.; **MOROCCO. Tanger-Tétouan:** Sidi Hassain, 10 km from Larache, 35º16’11”N 06º04’16”W, 19.III.1997, *Garilleti & Lara*; Ascent to Jbel Tassaot 35º15’16”N 5º09’23”W 10.VI.2004 *I. Draper & R. Medina*; **Taza:** Ascent to Jbel Tazzeka from Bab Bou Idir 34º03’14”N 4º10’19”W 21.VI.1997 **B. Albertos et al**.; **Rabat-Sale-Zemmour-Zaer:** near El-Harcha 33º28’15”N 6º06’49”W 22.VI.2000 *I. Draper, F. Lara & V. Mazimpaka*; **PORTUGAL: Bragança: [3]** Morais, 22.XI.2000, *Garilleti et al*.; **SPAIN.Almería: [1]** Sierra Alhamilla, 37º00’4”N 02°18’29”W, 22.VII.2006, *N.G.Medina*; **[4]** Sierra Alhamilla, 10.XI.2005, *R. Medina*; **Ávila:** Tolbaños, 40º44’19”N 4º33’06”W 1071 m, 21.III.2010, *N.G.Medina L51P2QI7*; **Cáceres:** Conquista de la

Sierra, 39º21'35"N 05º42'41"W, 26.III.2010, *N.G.Medina L26P23QI*; **Cádiz: [5] (holotype);** Algeciras, Sierra de la Luna, 36º06'24"N 05º32'23"W, 13.III.1998, *Guerra, Lara & Mazimpaka*; **Huelva:** Sierra de Aracena, Fuenteheridos, 10.II.1998, *Lara et al.*; **Lugo:** Lamamarín, Chantada, 27.X.1995, *Albertos et al.*; **Madrid:** Loeches, 40°23'01"N 3°26'49"W, 24.X.2003, *Albertos, Cortés & R.Medina;* **[2]** Cadalso de los Vidrios, 25.III.2006, *B. Estébanez & N. G. Medina*; **Toledo:** Los Navalmorales, 39º40'35"N 4º37'30"W 973 m, 8.III.2010, *N.G. Medina L41P14QI*; s/n Navalcán, 40º03'16"N 5º07'44"W 973 m, 10.III.2010, *N.G.Medina L43P1QI6*; **Santa Cruz de T.I.rife: [6]** La Gomera, Chipude, La Fortaleza, 28°06'01"N 17°16'35"W, 5.II.2005, *Lara;*

***Orthotrichum coulteri*. U.S.A. California:** Alameda Co., Berkeley, .X.1892, Howe (ny); Berkeley Campus, 18.IX.2008, *Lara & Garilleti*; **[2] [3] [4] [5]** Wildcat Canyon, 20.IX.2008, *Lara, Garilleti & Norris*; Contra Costa Co., **[6]** Gorge trail toward lake Anza. Tilden Regional Park, 29.XI.2006, *Norris 109508*, (UC-1917203); Monte Diablo State Park, 37º53'00"N 121º58'00"W, 980 ft., 4.I.2004, *Shevock 24500, Thayer & Bartosh*; Lake Co., Boggs Mountain, T11N R8W sec11, 3.IV.2010, *Toren 9733*; West base of Cobb Mountain, T11N R8W sec10, 3.IV.2010, *Toren 9732*; Los Angeles Co. Channel Islands, Santa Catalina Island, 33º23.281'N 118º23.974'W, 4.V.2010, *Carter 5757b* ; .Marin Co.., Olompali State Park, 38º09.4'N 122º34.5"W, 21.IV.2007, *Norris 109891 & Hillyard;* Monterey Co., Hastings Natural History Reservation, 3.III.1951, *Anonymous* (UC-1932399); Andrew Molera State Park, 36º16'56"N 121º50'02"W, 30 m, 4.VII.2003, Kellman 3063; San Francisco Co., Panhandle, 14.II.2000, *Shevock 18930* (UC-1741966); Upper Market area, 37º44'50.9"N 122º26'22.9"W, 24.I.2010, *Toren 9726b*; Forest Hill District, 37º45'40.5"N 122º26'24.3"W, 27.II.2010, *Toren 9727*; San Luis Obispo Co., Montana de Oro State Park, 35º16'N120º16'W, 25.XII.1982, *Norris 68193*; San Mateo Co., Wunderlich County Park; 122º 16' W, 37º 24' N, 600-800 ft, 4.III.1990, *Whittemore 347*1 (MICH);Santa Barbara Co. Channel Islands, Santa Cruz Island, 34º00.160'N 119º42.621'W, 18.V.2010, *Carter 4610*; Santa Clara Co., entrance to Henry W. Coe State Park, 37º06'N 121º28'W, 20.IV.1999, *Whittemore 6768 & Briggs* (CAS-1016718); North shore of Lake Grant, 37º21'N 121º43'W, 8.IV.1997, *Whittemore 6066*, (CAS-993134); Santa Cruz Co., **[7]** 37º12'02"N 122º02'38"W, *Kellman 2602* (CAS-1043175);Sonoma Co., gate to C. Adams Ranch, 12.II.1951, *Koch 3557* (UC-67435); Sonoma Co. **[1]** above Lake Sonoma at Skaggs Springs Road, 38º42'N 123º 01'W, 6.II.1990, *Norris 86911* (UC).

***Orthotrichum cucullatum*. U.S.A. California:** Alameda Co., **[1]** Berkeley, Bay View Place, 9.X.2008, *F. Lara*;**[7]** Berkeley, Fairlawn Dr, 22.IX.2008, *F. Lara*; **[5]** Berkeley, Cedar St., 30.IX.2008, *Lara & Garilleti*; **[2] [3]** Wildcat Canyon, 37º56'01"N 122º17'44"W, 20.IX.2008, *F. Lara, R. Garilleti & D. H. Norris*; Contra Costa Co., **[6]** trail on south shore of Briones Resevoir, 1.XI.2003, *Norris 106515 &Hillyard* (UC-1768584); Fresno Co., about 1 mile east of Monterey County line, 36º09'N 120º38'W, 5.IV.1995, *Norris 85107* (UC); Los Angeles Co., Arroyo Seco, near Sycamore Grove Park, 15.V.1926, *Moxley 1139* (NY); Channel Islands, Santa Catalina Island, 33º23.281'N 118º23.974'W, 4.V.2010, *Carter 5757a*; Madera Co., 4 miles south of O'Neal's, 37º05'N 119º42'W, 2.I.1994, *Norris 81851* (UC-1918187); Merced Co., Merced, Bear Creek Drive, 31.XII.1925, *Howell* (COLO-196626); Monterey Co., 28.III.1903, *Heller 6470* (NY); Garrapata State Park, 36º26'49"N 121º55'20"W, 14.VI.2003, *Kellman 2957* (CAS-1045418); Orange Co., **[10]** Cleveland National Forest, 33º44'51"N 117º35'00"W, 14.XI.2008, *Lara, Garilleti & Shevock*; Placer Co., **[8]** along Philip Rd., NW of Roseville, 1.III.2002, *Norris 103798* (UC-1760035); San Francisco Co., Presidio of San Francisco, 37º47'55"N 122º27'35"W, 20.V.2000, *Shevock 19368*, (CAS-1016500); Jefferson Square, 37º46'53"N 122º23'35"W, 5.II.2000, *Shevock 18902* (CAS-1016301); San Mateo Co., **[4]** Año Nuevo State Reserve, 25.X.2008, *Lara & Garilleti*; Santa Barbara Co., **[9]** Los Padres Nt Forest, 35º04'23"N 120º03'29"W, 17.XI.2008, *F. Lara, R. Garilleti & J. Shevock*; along Sierra Madre Road, 35º06'N 120º04.5'W, 28.XII.1979, *Norris 55301* (UC); Channel Islands, Santa Cruz Island, 33º59.932'N 119º45.443'W, 20.II.2011, *Carter 5316;* Santa Cruz Co., Corralitos, Merk Pond, 36º58'15"N 121º48'35"W, 5.VII.1999, *Kellman 719* (CAS-718768); near Aromas, 36º54'07"N 121º36'18"W, 2.IV.2000, *Kellman 929* (CAS-1017506);

***Orthotrichum franciscanum*. U.S.A. California:** Alameda Co., **[1]** Berkeley, Fairlawn Dr, 22.IX.2008, *F. Lara*; **[4]** Wildcat Canyon Regional Park 37º56'01"N 122º17'44"W, 20.IX.2008, *Lara, Garilleti & Norris*; **[2]** Berkeley, Cedar st. to Scenic st., 30.IX.2008 *Lara & Garilleti;* Contra Costa Co., Lime Ridge County Reserve, 8.IV.2001, *Norris 101336* (UC-101336); Los Angeles Co., Santa Catalina Island, canyon east of Black Jack Campgroung, 33º22'00"N 118º24'00"W, 25.III.1975, *Shevock 4073 &Thorne* (UC-1741891); San Jose Hills, near Pomona, 15.II.1919, *Munz 2407*; Monterey Co., **[10]** Hopkins 23.IX.2007; Napa Co., St. Helena, Crane

Park, 5.III.1973, *Norris 48234* (UC); San Diego Co., **[7]** Santa Margarita Ecological Reserve, 33º25’48”N 117º11’27”W, *Kellman 1389* (CAS-718654); San Francisco Co., **[3]** Lafayette Park, 16.IV.2000, *Shevock 19242* (UC-1739290); **[5]** along 3rd st., 22.X.2000, *Shevock 20202* (UC-1741780); Santa Clara Co., Stanford University Campus, 13.VI.1962, *Hermann 17461* (MICH);

Materials used for DNA extraction not included in chapter IV.4.

***Orthotrichum alpestre*. SWITZERLAND. St. Gallen: [2]** Vilters, Alphütte Untersäss, 13.VI.1992, *Scäfer-Verwimp 15465*; **TURKEY. Gümüşhane: [3]** Road from Maçka to Torul, 40º39’15”N 39º22’00”E, 15.VII.2005, *F. Lara, R. Medina & Mazimpaka*; ***Orthotrichum euryphyllum*. U.S.A. California: [1]** Lassen Co., 21 mi North of Litchfield, 17.V.1972, *F. Hermann J24764* (DUKE); **[2]** Shasta Co., along Cassel-Fall River road, 3.II.1995, *D. H. Norris 84701* (UC); ***Orthotrichum norrisii*. USA. California:** Alameda Co., **[1]** Wildcat Canyon, 20.IX.2008, *F. Lara, R. Garilleti & D. H. Norris;* **[3]** Berkeley campus, 17.IX.2008, *F. Lara, R. Garilleti & D. H. Norris*; San Francisco Co., **[4]** San Francisco, Panhandle Park, 14.II.2000, *J. R. Shevock 18930* (UC-1741966); Orange Co., **[5]** Cleveland National Forest, 33º44’51”N 117º35’00”W, 14.XI.2008, *F. Lara, R. Garilleti & J. Shevock*; Riverside Co., **[2]** San Jacinto Mts, Bay Tree Spring, 33º49’10”N 116º47’21”W, 16.XI.2008, *F. Lara, R. Garilleti & J. Shevock*; ***Orthotrichum tristriatum*. CHILE.** *Goffinet 5414* (CONN);

V.

Discusión general

> *"(...) los animales se dividen en (a) pertenecientes al Emperador, (b) embalsamados, (c) amaestrados, (d) lechones, (e) sirenas, (f) fabulosos, (g) perros sueltos, (h) incluidos en esta clasificación, (i) que se agitan como locos, (j) innumerables, (k) dibujados con un pincel finísimo de pelo de camello, (l) etcétera, (m) que acaban de romper el jarrón, (n) que de lejos parecen moscas."*
>
> Jorge Luis Borges, 1952
> El idioma analítico de John Wilkins

Utilidad taxonómica de los caracteres morfológicos

A continuación se discuten los caracteres morfológicos examinados, su variabilidad y su utilidad taxonómica en el marco de las especies tratadas a lo largo de esta tesis.

Colonias y hábito

Los gametóforos de las especies estudiadas presentan longitudes medias en relación con las descritas en el el género *Orthotrichum*. En todas ellas se pueden encontrar gametóforos de desarrollo modesto entre los 0.2 y los 0.4 cm, mientras que las longitudes máximas alcanzadas varían según las especies (hasta 0.5–1.0 cm, habitualmente). En algunos ejemplares de *Orthotrichum columbicum* y *O. scanicum*, los vástagos mejor desarrollados alcanzan o superan los 2 cm. En cuanto al hábito, todas las especies consideradas crecen en almohadillas compactas, algo más laxas en los ejemplares que alcanzan mayores tamaños.

Rizoides

En la mayor parte de las especies, las paredes de los rizoides son lisas y sólo ocasionalmente rugosas. Entre las especies estudiadas, únicamente en *Orthotrichum persimile* se han encontrado rizoides exclusivamente rugosos. Éste no es un carácter empleado en la taxonomía de *Orthotrichum* aunque sí hay antecedentes de su uso en el género *Zygodon* (Calabrese et al., 2002). En cuanto a su disposición en la planta, los rizoides se restringen en la mayor parte de los casos a las zonas basales de los gametóforos y sólo excepcionalmente aparecen en regiones más apicales. La ascendencia y persistencia de los rizoides en zonas superiores del gametofito es característica de *O. columbicum, O. consimile* y *O. confusum*. En cuanto a la presencia de yemas rizoidales, éstas se han observado de forma ocasional en *O. consimile, O. confusum, O. coulteri* y *O. pilosissimum*.

Caulidios

Muestran en general una sección subpentagonal (excepto en *O. comosum*, donde puede ser también redondeada), diferenciada invariablemente en una esclerodermis de 1-3 capas de células con paredes engrosadas que cubren un parénquima.

Pelos axilares

En la mayor parte de los casos son filiformes o levemente claviformes y constan de una serie de entre 3 y 7 células, de las cuales una o dos (las basales) están coloreadas y el resto son hialinas. Habitualmente miden entre 75 y 200 µm y no presentan una variabilidad interespecífica significativa, con la notable excepción de *Orthotrichum pilosissimum*. Como se puso de manifiesto en IV.3, esta especie se caracteriza por la posesión de abundantes pelos axilares de una longitud extraordinaria (hasta 1.8 mm) que llegan a recubrir el caulidio a modo de tomento e incluso sobresalir ocasionalmente de entre los filidios, como se aprecia al observar la planta a la lupa. Estos pelos axilares son excepcionales no sólo por su longitud, sino por estar constituidos, especialmente en la porción basal, por varias series de células y, a menudo, agruparse en fascículos.

Filidios

Los filidios presentan una alta variabilidad intraespecífica que puede apreciarse frecuentemente incluso dentro de una misma planta, por lo que las observaciones deben tener en cuenta varios de ellos procedentes de individuos diferentes y, a ser posible, que se encuentren completamente desarrollados. A continuación se analiza la utilidad taxonómica de los filidios en relación con su posición en estado seco, forma, tamaño, conformación del margen y del ápice, grosor y areolación de la lámina y nerviación.

Los filidios se disponen adpresos sobre el caulidio en seco y adquieren una posición erecto-patente en húmedo. Cuando la planta se halla deshidratada, el aspecto de los filidios puede resultar informativo, ya que éstos pueden ser más o menos rectos (en la mayoría de las especies) o variablemente flexuosos (*Orthotrichum columbicum, O. consimile, O. confusum, O. persimile* y *O. pilosissimum*).

La forma de los filidios es un aspecto significativo que necesita valorarse cuidadosamente, sin olvidar la variabilidad propia de cada individuo, y procurando examinar filidios que hayan completado su desarrollo. Generalmente los filidios maduros de *Orthotrichum* presentan un contorno linear-lanceolado u ovado-lanceolado, con una base ensanchada y una lámina que se estrecha gradualmente hacia el ápice. En cuanto a su tamaño, en las especies descritas los filidios oscilan entre los 1.2–3.5 mm de longitud y los 0.2–1.0 mm de anchura. Pese a la mencionada variabilidad, la forma y el tamaño de los filidios de un espécimen valorados en su conjunto, resultan útiles para orientar la identificación.

Las características del margen de los filidios se aprecian mejor en una sección transversal, preferiblemente hacia la zona central de la lámina. Por norma general el margen de los filidios es recurvado desde la base del mismo hasta el tercio apical e incluso casi hasta el ápice, y las variaciones de esta situación resultan taxonómicamente informativas para las especies que las presentan. Así, en *Orthotrichum persimile* es habitual que uno o ambos márgenes sean planos, mientras que en *O. franciscanum* y *O. pilosissimum* dicho margen es característicamente revoluto hacia la parte central de la lámina.

El ápice de los filidios ha resultado manifestar algunos de los caracteres más relevantes en el marco del presente estudio. Sin embargo, es bien conocido que la variabilidad de los ápices es muy elevada, incluso dentro de un mismo ejemplar (Lewinsky, 1993; Vitt, 1973). Hay que tener en cuenta que en cualquier espécimen aparecen hojas con ápices poco diferenciados, que no muestran los caracteres que se han considerado diagnósticos de la especie. Por ello, la descripción de los ápices debe atender a la observación sintética del espectro de variaciones presentes en la muestra.

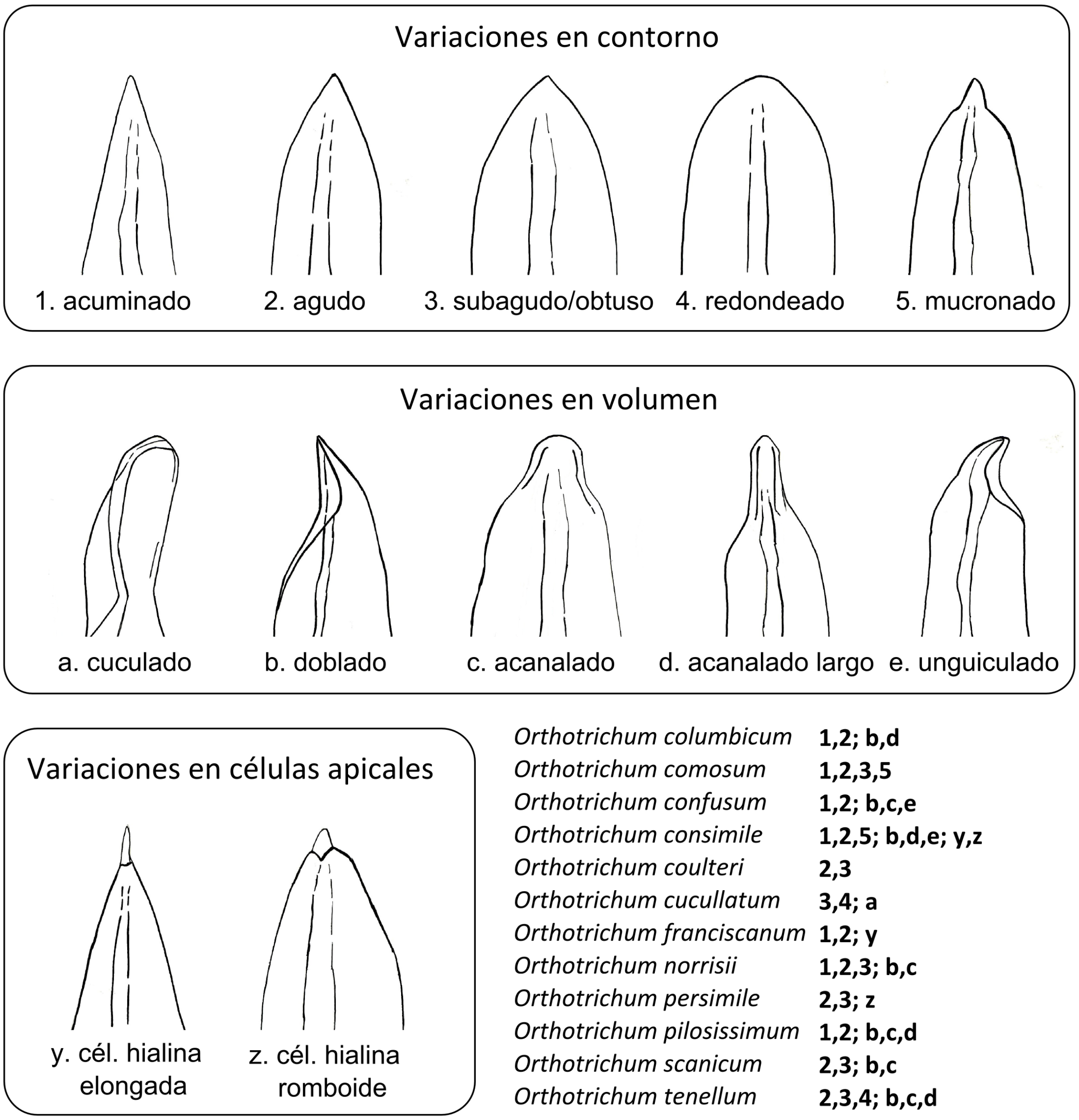
Podría decirse que la morfología del ápice del filidio se explica en función de tres variables: el contorno, el margen y la areolación (figura V.1).

El contorno del ápice se refiere simplemente al tipo de ángulo o forma en los que acaba el extremo del filidio. Entre las especies estudiadas hay ápices redondeados, obtusos, más o menos agudos e incluso acuminados, y es habitual que un mismo taxón presente cierta variación en este aspecto. La presencia de mucrones y súbulas, que a su vez pueden ser rectos o unguiculados, constituye otra alteración del contorno del ápice.

Aunque el margen de los filidios es, como se ha dicho, habitualmente recurvado, su comportamiento en la región apical varía notablemente. El margen del ápice puede ser plano, o como ocurre frecuentemente, incurvo en uno o ambos lados, produciendo dobleces o canales muy característicos que dan volumen al ápice.

Además, en ocasiones las células del ápice presentan diferenciaciones con respecto a las del resto de la lámina. Concretamente, entre las especies objeto de esta tesis, algunas manifiestan con cierta frecuencia una o varias células hialinas apicales de morfología y tamaño variables.

Atendiendo a la combinación de estos factores, se distinguen cinco tipos fundamentales de morfologías apicales en las especies estudiadas y se señala cuáles la presentan de forma característica. Consúltese también la figura V.1 en la que se recoge de forma más amplia la variabilidad mostrada por cada taxón.



Orthotrichum columbicum	**1,2; b,d**
Orthotrichum comosum	**1,2,3,5**
Orthotrichum confusum	**1,2; b,c,e**
Orthotrichum consimile	**1,2,5; b,d,e; y,z**
Orthotrichum coulteri	**2,3**
Orthotrichum cucullatum	**3,4; a**
Orthotrichum franciscanum	**1,2; y**
Orthotrichum norrisii	**1,2,3; b,c**
Orthotrichum persimile	**2,3; z**
Orthotrichum pilosissimum	**1,2; b,c,d**
Orthotrichum scanicum	**2,3; b,c**
Orthotrichum tenellum	**2,3,4; b,c,d**

FIG. V.1. Variabilidad en el ápice de los filidios de las especies estudiadas sintetizada a partir de las variaciones de su contorno, del volumen causado por la morfología del margen apical y de la presencia de células hialinas apicales. Se incluye cuáles de estas variaciones han sido observadas en cada una de las especies. (Dibujos por R. Caparrós)

1. Ápices de margen plano, subagudos, indiferenciados (es el caso de *Orthotrichum coulteri*).

2. Ápices redondeados y cuculados (*Orthotrichum cucullatum*).

3. Ápices de margen plano acabados en un pequeño número de células hialinas diferenciadas, bien relativamente pequeñas, no mucho mayores que las del resto de la lámina (*Orthotrichum persimile*), o bien característicamente elongadas (*O. franciscanum*).

4. Ápices de margen plano, elongados, acabados en un mucrón o una súbula corta y recta (*Orthotrichum comosum*) o bien más o menos ondulada e incluso unguiculada (*Orthotrichum consimile* y *O. confusum*).

5. Ápices con márgenes incurvados de manera variable, dando lugar a canales o dobleces. Esto ocurre con frecuencia en un buen número de las especies estudiadas, aunque no siempre formando el mismo tipo de diferenciaciones. Puede ocurrir que uno de los márgenes se incurve, generando una silueta característicamente unguiculada (*Orthotrichum confusum* y *O. consimile*), o bien que se formen dobleces sencillos (*Orthotrichum norrisii* y *O. scanicum*). Cuando ambos márgenes se incurvan, el ápice muestra un característico canal (también aparece con frecuencia en *O. norrisii* y *O. scanicum* y es especialmente característica de *O. tenellum*). En ciertas especies este canal está característicamente elongado, y puede ser incluso quebradizo (*Orthotrichum columbicum* y *O. pilosissimum*).

Las láminas de los filidios son típicamente unistratas y ligeramente aquilladas. Se ha observado ocasionalmente la presencia de bandas bistratas en la lámina de algunos especímenes de *Orthotrichum coulteri* y *O. pilosissimum*.

El nervio es bastante constante en sus características dentro de las especies consideradas en el presente estudio. Se trata siempre de nervios simples que terminan bajo el ápice. Muestran una sección reniforme en la que se aprecian dos células guía.

La areolación de las células basales y el grosor de sus paredes son rasgos relevantes en la taxonomía del género en su conjunto, pero no han resultado informativos en el presente estudio por su elevada variabilidad intraespecífica. Las células de la parte central y apical de la lámina del filidio son isodiamétricas o algo elongadas. El tamaño de las mismas no ha sido útil para la diferenciación de especies en el grupo estudiado, al igual que el grosor de las paredes celulares. Las células superiores son frecuentemente papilosas, con 2-3 papilas en cada cara. El grado de desarrollo de estas papilas es a veces muy llamativo, lo que le da a la planta un tono glauco, visible incluso cuando se observacon lupa. Se han encontrado ejemplares de *Orthotrichum scanicum* y *O. pilosissimum* con papilas ramificadas de hasta 15 µm de longitud. Aunque en ocasiones este tipo de papilas se han empleado taxonómicamente (Lewinsky-Haapasaari, 1996; Goffinet et al., 2007), hay indicios de que especies muy diversas son capaces de desarrollarlas, por lo que su utilización con fines taxonómicos en este género debe hacerse con mucha cautela (Medina et al., 2010). En el contexto de esta memoria, la ausencia de papilas muy desarrolladas y ramificadas tan sólo resulta de utilidad para distinguir *O. comosum* de *O. alpestre* (especie que sí las presenta de forma regular).

Yemas

Habitualmente oscilan entre 75 y 250 µm de longitud y están compuestas por células marrones o verdes dispuestas en serie. Nunca presentan tabiques longitudinales y raramente son ramificadas. Entre los taxones considerados en el estudio sólo se han observado yemas ramificadas de forma habitual en *Orthotrichum pilosissimum*, que además pueden ser muy largas (hasta 600 µm). De forma ocasional se han encontrado yemas de este tipo en *O. consimile* y *O. confusum*. En la mayor parte de las ocasiones, estos propágulos se forman a partir de células de la lámina de los filidios, aunque se han detectado casos de rizoides propagulíferos en *O. pilosissimum*, *O. consimile* y *O. confusum*. La presencia y abundancia de yemas es muy variable dentro incluso de una misma especie. Algunos especímenes las producen en abundancia, mientras que en otros son relativamente raras, lo que sugiere que puede haber mecanismos de respuesta a factores ambientales que determinen su profusión. Únicamente en *O. columbicum* no se han observado nunca yemas.

Sexualidad

La condición cladautoica es con diferencia la más frecuente entre las especies estudiadas: las ramas masculinas (con perigonio apical) se ramifican por debajo del ápice, originando una rama que puede ser masculina o femenina. Esta ramificación no siempre se distingue con claridad, pudiendo llegar a la errónea conclusión de que todos los perigonios y periquecios pertenecen a un mismo módulo de crecimiento, por lo que es fácil confundir un espécimen cladautoico con uno verdaderamente goniautoico. Todos los taxones estudiados son cladautoicos, excepto *Orthotrichum comosum* y *O. scanicum*, que pueden ser goniautoicos o cladautoicos, y *O. norrisi* que es goniautoico.

Vagínula

La vagínula es un órgano al que no siempre se le ha prestado suficiente atención, pero que alberga varios caracteres de especial relevancia taxonómica en el conjunto de taxones tratados en esta memoria. Las variaciones significativas son las que afectan a la longitud y forma por un lado, y a la pelosidad por otro. Las vagínulas tienen habitualmente forma cilíndrica o troncocónica y una longitud de entre 0.3 y 0.7 mm. En *Orthotrichum confusum* y *O. pilosissimum* resulta relevante la presencia de vagínulas cilíndricas o fusiformes, de longitudes que con frecuencia superan 1 mm de longitud.

La presencia o no de pelos en la vagínula y, en su caso, la morfología de los mismos, es una fuente muy significativa de caracteres taxonómicos. Aunque se trata de un carácter con notable estabilidad intraespecífica, debe tenerse en cuenta que una pequeña proporción de los taxones estudiados que suelen presentar vagínulas desnudas pueden mostrar algún pelo aislado y viceversa. Estas excepciones deben contemplarse dentro de la variabilidad propia de cualquier carácter y en caso de duda deben examinarse varios pies del mismo ejemplar. Los taxones con vagínulas típicamente desnudas no desarrollan vagínulas densamente pelosas ni a la inversa.

Presentan vagínulas típicamente desnudas *Orthotrichum columbicum, O. comosum, O. confusum, O. pilosissimum, O. scanicum* y *O. tenellum.* Por otra parte, en *O. consimile, O. franciscanum* y *O. norrisii* es habitual la presencia de pelos dispersos, mientras que *O. cucullatum, O. coulteri* y *O. persimile* desarrollan vagínulas netamente pelosas. En la mayor parte de los casos, los pelos están formados por una serie de células hialinas alargadas con paredes más o menos engrosadas. Ocasionalmente los pelos están formados parcial o totalmente por varias series de células alargadas (por ejemplo, en *O. norrisii*). En *O. consimile*, los pelos de la vagínula están formados mayoritariamente por características células cortas de paredes muy gruesas y papilosas.

Caliptra

La caliptra madura suele ser mitrada, oblongo-cónica a largamente campanulada y acabada en un pico terminal, generalmente de color más oscuro. Algunas especies con cápsulas más pequeñas (por ejemplo, *Orthotrichum franciscanum*, *O. comosum* u *O. cucullatum*) presentan caliptras oblongas, mientras que en otros casos (de forma característica en *O. columbicum*) las caliptras son largas y subcónicas.

La pilosidad de la caliptra y la morfología y situación de los pelos en el caso de que los presenten también tiene relevancia taxonómica. Al igual que se ha dicho en el caso de la pilosidad de la vagínula, es esperable encontrarse con casos excepcionales que se salgan de la norma y que son atribuibles a la variabilidad del carácter. Los pelos de la caliptra son similares a los pelos que aparecen en la vagínula, aunque más frecuentemente multiseriados y con paredes celulares engrosadas, a veces papilosas.

Mientras que las caliptras de *Orthotrichum columbicum* y *O. confusum* son típicamente desnudas, la mayoría de las especies tratadas presentan algún tipo de pilosidad. La variabilidad presente en estos casos es grande. *Orthotrichum coulteri* y *O. persimile* tienen caliptras típica y conspicuamente pilosas. *Orthotrichum comosum* y, en menor medida, *O. norrisii*, presentan caliptras con una característica coma apical de abundantes pelos.

Orthotrichum consimile posee pelos característicos, son muy gruesos, multiseriados y formados por células cortas de paredes celulares gruesas y fuertemente papilosas. Finalmente, *O. cucullatum, O. franciscanum, O. scanicum, O. tenellum* y *O. pilosissimum* tienen caliptras con pelos dispersos que a veces pueden ser muy escasos y cortos, difíciles de detectar si no es mediante observación microscópica e incluso, en el extremo de su variabilidad, caliptras totalmente desnudas.

Seta

La seta eleva la cápsula con una torsión antihoraria. La longitud de la seta y su relación con la de los filidios periqueciales determina el grado de emersión de la cápsula. En el conjunto de las especies consideradas en este estudio las setas más cortas son las de *Orthotrichum comosum* (0.3–0.6 mm) y las más largas corresponden a *O. columbicum* (2.5–5.5 mm). Todas las demás son intermedias, variando entre especies de cápsulas típicamente inmersas o emergentes (*O. cucullatum, O. franciscanum, O. norrisii, O. pilosissimum, O. scanicum* y *O. tenellum*) a variablemente exertas (*O. confusum, O. consimile, O. coulteri* y *O. persimile*).

Opérculo

Su morfología varía entre plano (*Orthotrichum norrisii*), subcónico (en la mayoría de los casos) y netamente convexo (*O. scanicum*). Su diámetro es relativamente variable en el conjunto de las especies estudiadas en función del desarrollo de la cápsula. Presenta siempre un rostro de longitud y grosor variable y habitualmente un anillo basal de color marrón, anaranjado o rojizo formado por células de paredes engrosadas. Este anillo es especialmente notable en *Orthotrichum columbicum* y *O. norrisii*, mientras que está prácticamente ausente en *O. scanicum*.

Cápsula

La cápsula en el género Orthotrichum reúne una serie de caracteres taxonómicamente significativos que conciernen a su forma, la areolación del exotecio, así como el tipo y posición de los estomas.

<u>Forma</u>. La forma de la cápsula varía tanto a lo largo de su desarrollo (especialmente durante la liberación de las esporas al quedarse vacía) como en función de su estado de hidratación. Sin embargo, en el contexto del presente estudio, el aspecto que resulta

más informativo es la forma que adquiere la urna seca en su madurez, ya que las formas juveniles son relativamente uniformes (fusiformes a subcilíndricas), al igual que cuando se hidratan (ovoide a piriforme). Las cápsulas maduras y secas son, por norma general, cilíndricas y algo constreñidas bajo la boca. Es significativa la excepción que constituye *Orthotrichum norrisii*, con cápsulas cilíndricas que no se constriñen. En el otro extremo se encuentran las cápsulas de *O. confusum* y *O. consimile*, que pueden mostrar morfologías urceoladas, y *O. pilosissimum*, que se contrae prácticamente en toda su longitud cuando se liberan las esporas. Todos los taxones considerados en este estudio muestran cápsulas con cuellos defluentes.

Exotecio. Las especies objeto del estudio tienen siempre cápsulas con 8 costillas longitudinales bien desarrolladas y un anillo de células isodiamétricas pequeñas alrededor de la boca de la cápsula. El tejido exotecial varía en el número de células que constituyen la anchura de las bandas exoteciales. Lo más frecuente es que las costillas estén formadas por 4–5 series de células diferenciadas, subcuadradas o rectangulares, de paredes engrosadas, localmente duplicadas por un tabique longitudinal. Sin embargo, no es siempre así: *Orthotrichum norrisii* y *O. scanicum* se caracterizan por tener bandas exoteciales estrechas, formadas sólo por dos series de células. Algunos especímenes de *O. coulteri* poseen costillas que pueden ser igualmente finas, aunque se trata de casos excepcionales.

Estomas. Como en el resto de los integrantes del subgénero Pulchella, los estomas de las especies estudiadas están siempre inmersos en el exotecio y cubiertos en mayor o menor medida por las células exoteciales adyacentes (criptoporos). El grado de recubrimiento de los estomas es un carácter taxonómico en el conjunto del subgénero, pero no resulta especialmente informativo en el contexto de este estudio, pues todos los taxones muestran estomas casi o totalmente cubiertos. La posición de los estomas a lo largo de la urna también es un rasgo taxonómicamente informativo en el subgénero que, en cambio, sí muestra clara variabilidad dentro del grupo de estudio. Habitualmente los estomas se encuentren mayoritariamente concentrados en la mitad basal de la urna, aunque sin llegar al cuello (*Orthotrichum columbicum, O. cucullatum, O. norrisii, O. pilosissimum, O. scanicum* y *O. tenellum*). Sin embargo, algunos taxones también tienen estomas en el cuello de la cápsula (*O. comosum, O. coulteri, O. franciscanum* y *O. persimile*). Por su parte, *O. confusum* y *O. consimile* presentan los estomas principalmente hacia el centro de la urna, y sólo de forma excepcional algunos aparecen en zonas más inferiores de la urna.

Peristoma

Exostoma. En todos los taxones considerados los dientes están fusionados formando ocho pares, son triangulares, permanecen recurvados en seco y reaccionan higroscópicamente cerrando la boca de la cápsula cuando se humedecen (esporosis xerocástica). Generalmente son de color amarillento o blanquecino, ocasionalmente anaranjado. La OPL exostomial, más gruesa que la PPL, presenta una notable diversidad de ornamentaciones. En la parte más basal es habitual observar un retículo continuo que puede ser relativamente liso como en algunos ejemplares de *Orthotrichum consimile*, o variablemente papiloso como en *O. tenellum*. Generalmente, la ornamentación se vuelve más marcada hacia el extremo apical de la OPL, donde, además de poder observarse papilas uniformes, es habitual que éstas se unan formando líneas vermiculares que pueden ser predominantemente longitudinales (por ejemplo en *O. norrisii, O. franciscanum, O. scanicum*), transversales (como ocurre a veces en *O. pilosissimum*), radiales (*O. confusum*) o una combinación de ellas. La OPL del exostoma de *O. comosum* puede presentar líneas transversales en la parte basal y longitudinales en el ápice del diente. En el contexto del presente estudio, la ornamentación de la OPL presenta bastante variabilidad intraespecífica y posee un limitado valor diagnóstico, aunque la ornamentación con líneas vermiculares en esta región ayuda a distinguir *O. comosum* de *O. alpestre* (que no las manifiesta). La PPL exostomial es habitualmente lisa o casi lisa, con la excepción de *O. columbicum* y *O. scanicum*, que presentan en esta capa líneas longitudinales definidas. Se han encontrado de forma ocasional otros exostomas con cierta ornamentación apical en su PPL: papilas y/o líneas en *O. comosum* y *O. tenellum* y líneas radiales en *O. cucullatum*. La reabsorción de las paredes periclinales del ápice de los dientes, mientras se mantienen intactas las anticlinales, es frecuente en ejemplares de *O. franciscanum*, cuyos dientes del exostoma adquieren en esas ocasiones un aspecto truncado o fragmentado.

Endostoma. Los endostomas mejor desarrollados aparecen en *Orthotrichum columbicum* y *O. scanicum*, en los que se mantienen los dieciséis segmentos, si bien los intermedios suelen ser algo más cortos. Los segmentos principales suelen ser al menos parcialmente biseriados, mientras que los intermedios son frecuentemente uniseriados. En el resto de los casos el endostoma mantiene ocho segmentos que se alternan con los pares de dientes del exostoma. Por regla general, las superficies del endostoma no presentan ornamentaciones muy patentes en las especies consideradas en el estudio y son frecuentes los especímenes que carecen de ornamentación en los segmentos. La PPL endostomial puede ser rugosa o papilosa si la PPL del exostoma presenta algún tipo de ornamentación, e igualmente se pueden apreciar papilas sutiles y líneas en la IPL de distintas especies. Es digna de mención la existencia de endostomas muy papilosos en ejemplares de *O. scanicum* y *O. tenellum*, como parte de la variabilidad de este carácter.

La membrana conectiva no está bien desarrollada en la mayoría de las especies tratadas, pues normalmente es baja y en ocasiones discontinua. Constituyen la excepción las especies con endostomas de dieciséis segmentos (*Orthotrichum columbicum* y *O. scanicum*), así como *O. consimile* y *O. persimile*.

Esporas

Las esporas de los taxones estudiados son subesféricas y papilosas. El diámetro de las esporas es típicamente de 12–18 µm, aunque variable tanto intra- como interespecíficamente. En ocasiones, el intervalo de estos tamaños puede aportar información adicional para la discriminación de algunas especies, por ejemplo, *Orthotrichum consimile* (8–13 µm) de *O. persimile* (15–17 µm).

Considerados individualmente, gran parte de los caracteres enumerados presentan una variabilidad que no pocas veces complica su manejo taxonómico. Concretamente, algunos manifiestan una gran variación intraespecífica (incluso dentro del mismo individuo), mientras que otros son compartidos por muchas especies distintas. Esto explica en parte las dificultades que los integrantes de este género suelen presentar a los no expertos. Como ocurre también en otros grandes géneros de musgos (p.e. *Bryum, Grimmia*, etc), en ningún caso un carácter aislado debe usarse para una identificación adecuada; el examen de la combinación de caracteres exclusiva para cada taxón junto a una cuidadosa valoración de algunos caracteres secundarios deben ser las directrices a seguir para una identificación óptima.

Contribución del enfoque integrativo

El estudio de los supuestos taxones disyuntos de *Orthotrichum consimile* y *O. tenellum* a través de la taxonomía integrativa ha revelado la existencia de una diversidad específica que había pasado en gran parte desapercibida. Se han identificado un total de once especies: ocho de ellas restringidas al oeste de Norteamérica, dos presentes en el oeste de la región Paleártica y únicamente una distribuida en ambas regiones.

Todos estos taxones son plenamente distinguibles, tanto por una combinación única de caracteres como por constituir clados robustos en las reconstrucciones filogenéticas efectuadas. Sin embargo, ha sido la síntesis de ambas aproximaciones la que ha permitido alcanzar una solución robusta a la vez que pragmática y accesible.

Desde un punto de vista exclusivamente morfológico, se podrían haber alcanzado en un momento dado las mismas conclusiones. No obstante, sin un método independiente de comprobación de la hipótesis morfológica, en este caso aportado por la filogenia molecular, las relaciones entre los taxones habrían permanecido ocultas y el criterio experto a la hora de efectuar la delimitación habría quedado sin valorarse críticamente. Además, la sutil segregación entre *Orthotrichum consimile* y *O. confusum* habría permanecido, probablemente, sin detectarse.

Del mismo modo, la aproximación mediante filogenia molecular habría tenido éxito revelando la diversidad subyacente en los problemas abordados, pero sólo en el caso improbable de que se incluyeran por azar extractos de todos los morfotipos estudiados. El problema adicional de esta aproximación únicamente molecular reside en la ausencia de soluciones taxonómicas, y, por lo tanto, en la inaccesibilidad práctica de este conocimiento para investigadores sin posibilidad de obtener datos moleculares. Este problema es recurrente en muchos trabajos filogenéticos actuales, que basándose en la detección de estructuras complejas en la filogenia, toman decisiones taxonómicas sin que haya una revisión crítica de la morfología por parte de especialistas. Es posible que una parte sustancial de lo que se describen como especies crípticas pudiera beneficiarse de estudios integrativos como los aquí presentados.

Los resultados obtenidos sugieren además cierta cautela a la hora de sacar conclusiones generales sobre la biogeografía de especies de briófitos. En el reducido marco de las especies objeto de estudio se aprecian patrones biogeográficos diversos: se incluyen algunas especies que tienen distribuciones disyuntas transcontinentales (*Orthotrichum columbicum*, aunque también se ha puesto de manifiesto en el caso de *O. pulchellum*), así como otras con distribuciones más restringidas e incluso endémicas (como ocurre con algunas especies de California).

Implicaciones filogenéticas

Aunque la filogenia y la clasificación infragenérica de *Orthotrichum* s.l. no forman parte de los objetivos de esta tesis, los resultados obtenidos anticipan algunas implicaciones de interés sobre este aspecto, que aún permanece en gran parte sin investigar. Los resultados filogenéticos obtenidos son coherentes con la aproximación general de Goffinet et al. (2004) y sugieren que los *Orthotrichum* de estomas inmersos forman un grupo monofilético segregado de los de estomas superficiales (a su vez estrechamente relacionados con *Ulota*) y del género *Nyholmiella*. Como se puso de manifiesto en la discusión del capítulo IV.5, las filogenias obtenidas son útiles en cuanto a la delimitación específica, pero la robustez de muchos clados es moderada o baja y la topología obtenida por los marcadores cloroplásticos presenta incongruencias respecto a la obtenida por los marcadores nucleares. Independientemente de sus posibles causas (por ejemplo, eventos de reticulación), esta circunstancia advierte de que la resolución adecuada de la filogenia de este grupo puede requerir el complemento de otras fuentes de información, como los microsatélites (Vanderpoorten & Shaw, 2010).

A pesar de esta incongruencia topológica, y teniendo en cuenta que la selección de taxones aquí tratada es sólo una pequeña parte de toda la diversidad específica incluida entre los *Orthotrichum* de estomas inmersos, los resultados de los capítulos IV.4 y IV.5 sugieren cierta estructura filogenética en este grupo. A continuación se comentan estos resultados brevemente en el contexto de la clasificación infraespecífica de Lewinsky (1993).

Concretamente, los datos cloroplásticos agrupan como un clado bien definido a *Orthotrichum alpestre*, *O. patens* y *O. stramineum* (clado C en el capítulo IV.5), un grupo de especies cuya afinidad morfológica es bien conocida (Lara et al., 2008). Los datos nucleares disponibles sólo permiten confirmar que *O. alpestre* no presenta afinidades estrechas con las otras especies estudiadas, puesto que no se han secuenciado marcadores nucleares de *O. patens* ni de *O. stramineum*. Todas estas especies pertenecerían al subgénero Pulchella, sección Diaphana. El otro clado con fuerte apoyo estadístico (D en IV.5) es el constituido por *O. consimile* y especies relacionadas (*O. columbicum, O. pulchellum, O. confusum* y *O. aequatoreum*) así como por *O. anomalum* y *O. cupulatum*. Este clado se mantiene de forma coherente tanto en los resultados del genoma cloroplástico como en los del nuclear. Los taxones de este linaje se ajustarían en líneas generales a la suma del subgénero *Orthotrichum* y la sección *Pulchella* del subgénero *Pulchella* (Lewinsky, 1993). El resto de las especies consideradas (clados A y B, que constituyen el grueso del subgénero *Pulchella*, sección *Diaphana*) no presentan afinidades tan claras y su posición en la filogenia e incluso la situación de determinadas especies varían en función del genoma.

Varios trabajos de reciente publicación (Sawicki et al., 2009a; 2009b; 2010; 2012) se han centrado en la filogenia del género *Orthotrichum* s.l. y en la revisión de su clasificación infraespecífica. En líneas generales coinciden con lo expuesto anteriormente en lo que respecta a la división clara entre los *Orthotrichum* de estomas superficiales, el género *Nyholmiella* y los *Orthotrichum* de estomas inmersos. Más allá de este nivel, son muy pocas las conclusiones sobre las relaciones interespecíficas que se manifiestan en las publicaciones mencionadas, puesto que los clados basales presentan escaso apoyo estadístico. Este mismo problema se manifestó en los estudios preliminares de esta tesis y se solventó en gran medida con la combinación del mayor número posible de marcadores de ambos genomas. Los resultados obtenidos para los *Orthotrichum* de estomas inmersos en Sawicki et al. (2012) se basan en secuencias nucleares ITS (desestimadas en esta tesis), un único marcador cloroplástico (*trnH-psbA*) y técnicas de análisis de patrones de bandas ISJ (Intron-exon Splice Junctions) e ISSR (Inter-Simple

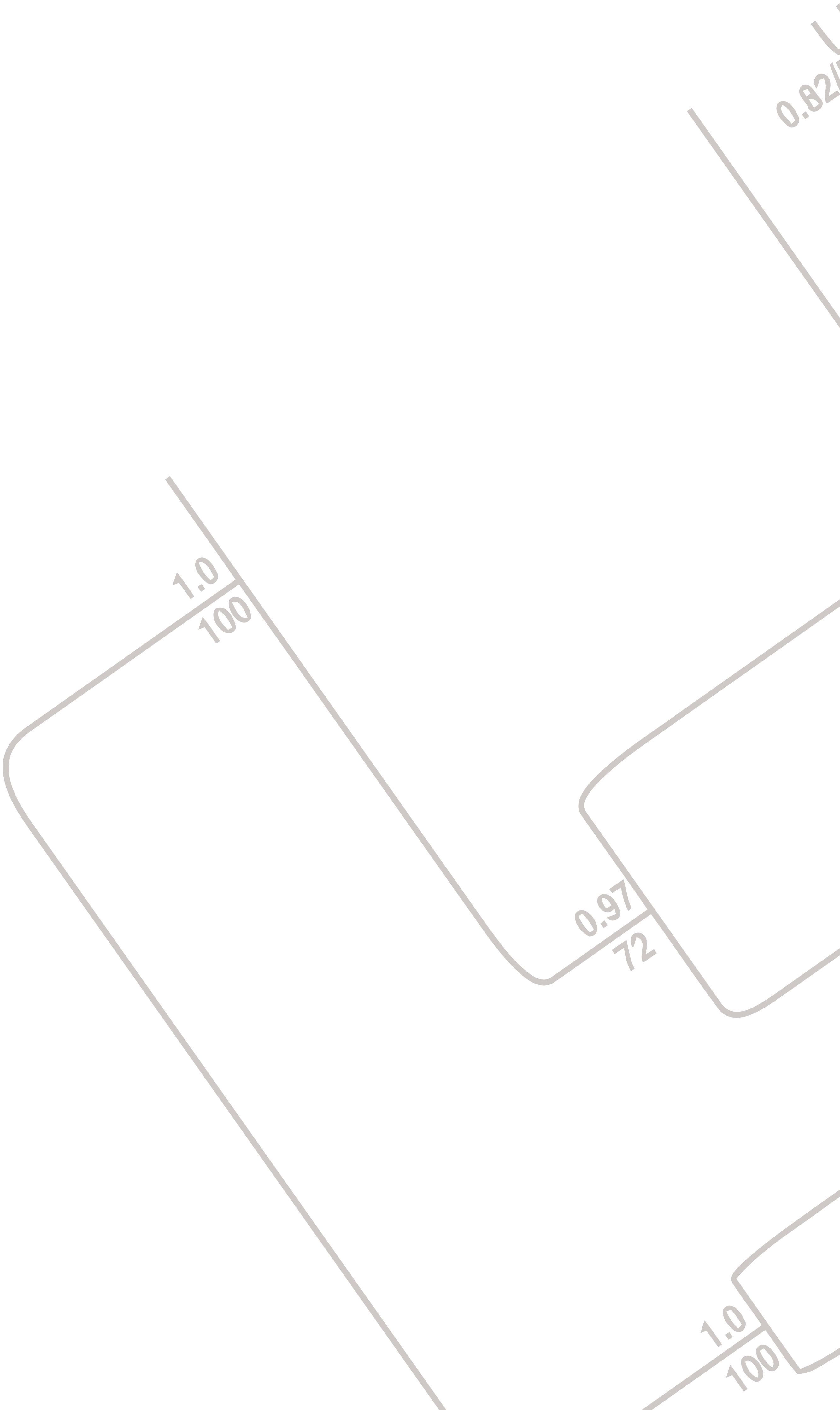
Sequence Repeats). Es difícil concluir si la falta de resolución de una posible clasificación infragenérica (que, aunque de forma parcial se puede inferir en los resultados de esta tesis) se debe a la necesidad de conseguir una señal filogenética suficientemente fuerte o a una incoherencia entre las señales de los marcadores usados por Sawicki y colaboradores y los que se han presentado en esta memoria. En cualquier caso, una respuesta definitiva y robusta a la filogenia de este género prolijo y diverso (con sus correspondientes consecuencias taxonómicas y nomenclaturales) requerirá un esfuerzo adecuado tanto en la elección de una muestra representativa de su diversidad (morfológica y geográfica) como en la obtención de datos moleculares suficientes.

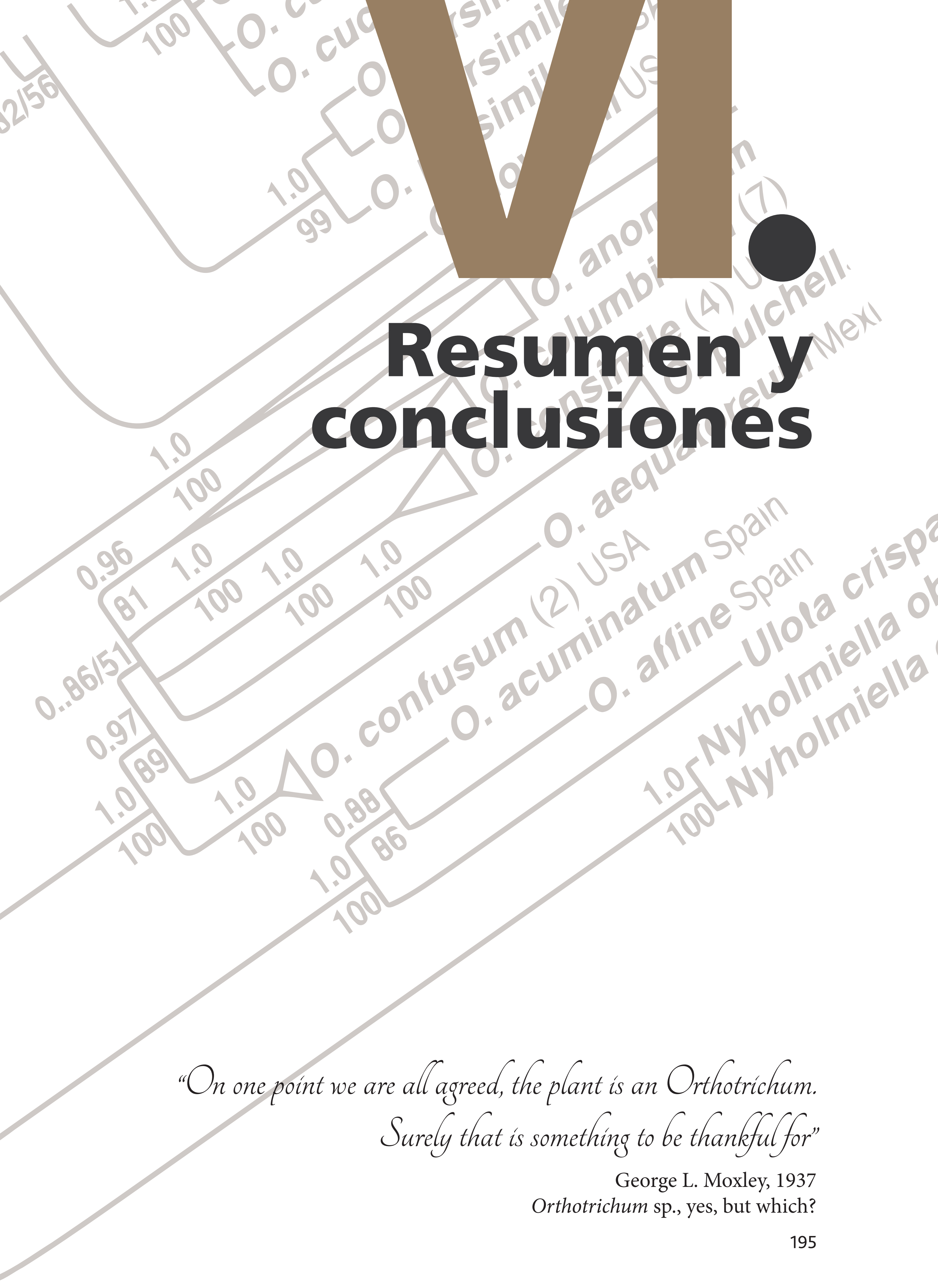
Vías de ampliación

La complejidad descubierta en especies de *Orthotrichum* que se consideraban simplemente ampliamente distribuidas y muy variables plantea la cuestión de hasta qué punto pueden darse casos parecidos en otros taxones de la familia *Orthotrichaceae* y, por extensión, en el resto de los briófitos. Ello sugiere la necesidad de plantear otros estudios en la misma línea enfocados a resolver de forma más rigurosa y robusta los problemas taxonómicos que aún persisten en este grupo de briófitos.

Hay varias especies de *Orthotrichum* que, por englobar una variabilidad morfológica muy amplia y por presentar disyunciones transcontinentales, son buenas candidatas a albergar una diversidad todavía sin descubrir. Algunos ejemplos podrían ser otras especies presentes en Norteamérica y Europa, como *Orthotrichum alpestre*, e incluso algunas con distribuciones más amplias aún, como *Orthotrichum diaphanum* Schrad. ex Brid., presente en Europa, África y ambos subcontinentes americanos. Sin embargo, la experiencia adquirida durante trabajos preliminares a los que se presentan en esta tesis indican que no siempre tienen por qué obtenerse resultados satisfactorios con la metodología que ha sido expuesta y, concretamente, con el uso de los marcadores moleculares empleados, que para muchos taxones no manifiestan una variabilidad interespecífica suficiente. El problema derivado de una escasa variabilidad interespecífica en el género *Orthotrichum* (en comparación con otros trabajos previos en briófitos) supuso un desafío para el desarrollo de este estudio desde el primer momento.

Concretamente, los estudios preliminares en *Orthotrichum pumilum* Sw. ex anon, *O. pallens* Bruch ex Brid. y especies afines mostraron una variabilidad escasa en las secuencias obtenidas para los loci que luego han resultado útiles en el presente estudio. Esta misma situación se acentúa aún más en especies de *Orthotrichum* de estomas superficiales, donde la variabilidad es muy escasa incluso entre especies que podrían suponerse poco relacionadas. Estos casos podrían abordarse mediante reconstrucciones filogenéticas no dicotómicas, sino reticuladas, como las redes tipo “*split network*” (e.g. Draper et al., 2007) o las redes de haplotipos (e.g. Hedenäs & Eldenäs, 2008). Además, cuando el objetivo del estudio es la delimitación específica, es especialmente recomendable complementar el análisis de secuencias con el uso de técnicas como RFLPs o microsatélites (Vanderpoorten & Shaw 2010). Mediante estas técnicas más finas también podrían resolverse las cuestiones filogeográficas derivadas del presente estudio, como la explicación del proceso de divergencia entre *Orthotrichum tenellum* y *O. norrisii*, la comprobación de la existencia de conectividad genética entre las poblaciones disyuntas de *O. columbicum* y la posible radiación simpátrica en la región de California.


0.82/
1.0
100
0.97
72
1.0
100



VI.

Resumen y conclusiones

“On one point we are all agreed, the plant is an Orthotrichum.
Surely that is something to be thankful for”

George L. Moxley, 1937
Orthotrichum sp., yes, but which?

Resumen

Esta tesis se ha realizado con el objetivo de aportar soluciones robustas e integrativas a determinados problemas taxonómicos en el seno del género *Orthotrichum*, subgénero Pulchella. Concretamente se han abordado tres núcleos conflictivos: *Orthotrichum scanicum* (una especie insuficientemente conocida pero incluida en la Lista Roja Mundial de briófitos amenazados) y los dos supuestos complejos taxonómicos de *Orthotrichum consimile* y *Orthotrichum tenellum* (consideradas ambas especies muy variables con distribución disyunta transcontinental en el oeste del Neártico y el Paleártico). Para ello se ha combinado, cuando ha sido posible, tanto una revisión morfológica en profundidad como una reconstrucción filogenética a partir de regiones de los genomas cloroplástico y nuclear.

En total se han estudiado más de 750 especímenes de herbario, en los que se han examinado los caracteres de relevancia taxonómica ya conocidos a la vez que se han buscado otros nuevos. Por otra parte se hizo una selección de especímenes de reciente recolección para extraer y amplificar los marcadores *atpB-rbcL, rps4, trnG* y *trnL-F* del genoma cloroplástico y *AdK* y *ort-Lfy* del genoma nuclear secuenciando en el proceso unas 308.000 pares de bases.

En el caso particular de *Orthotrichum scanicum*, se ha realizado una delimitación más precisa y una redescripción actualizada del taxón, discriminándola de especies morfológicamente similares (IV.2) y contextualizándola filogenéticamente (IV.4 y IV.5), para concluir que su estatus de amenaza global se debía en realidad a un conocimiento insuficiente de su variabilidad y distribución.

El estudio de la diversidad incluida en *Orthotrichum consimile* s.l. (capítulo IV.4) reveló la existencia de cuatro especies, tres de ellas restringidas a la región pacífica de Norteamérica (*Orthotrichum consimile* s.s., *O. confusum sp. nov.* y *O. persimile sp. nov*.) y una presente además en el continente europeo y que había sido sinonimizada (*O. columbicum*).

De forma análoga, el estudio del caso de *Orthotrichum tenellum* s.l. (capítulos IV.1, IV.3 y IV.5) puso de manifiesto la presencia de dos especies en el occidente del Paleártico (*O. tenellum* s.s. y *O. comosum sp. nov*.) y cinco en la región occidental de Norteamérica (*O. coulteri*, *O. norrisii sp. nov*., *O. pilosissimum sp. nov*., *O. cucullatum sp. nov* y *O. franciscanum sp. nov*.).

Conclusiones

1. Se aportan dos nuevos sinónimos:

Para *Orthotrichum scanicum*: *Orthotrichum holmenii*
Para *Orthotrichum pulchellum*: *Orthotrichum hendersonii*

2. Se descarta la sinonimia de *Orthotrichum coulteri* y *Orthotrichum tenellum*. Se reconoce la sinonimia de *Orthotrichum coulteri* y *Orthotrichum cylindrocarpum*, donde resulta prioritario el binomio *Orthotrichum coulteri*.

3. Se descarta la sinonimia de *Orthotrichum columbicum* y *Orthotrichum consimile*. Además, se pone de manifiesto que el concepto que generalmente se tiene de *Orthotrichum consimile* se corresponde en realidad con *Orthotrichum columbicum*.

4. Se describen siete especies nuevas para la ciencia:

Orthotrichum norrisii

Orthotrichum pilosissimum

Orthotrichum confusum

Orthotrichum persimile

Orthotrichum comosum

Orthotrichum cucullatum

Orthotrichum franciscanum

5. Se identifican los principales caracteres morfológicos que permiten la identificación de las especies tratadas en este estudio: el grado de desarrollo de los pelos axilares; la forma de los filidios; las diferenciaciones de su ápice y la curvatura de su margen; la longitud, forma y pilosidad de la vagínula; la forma y pilosidad de la caliptra; la forma del opérculo; la longitud de la seta; la forma de la cápsula seca, vacía y madura; la constitución de las bandas exoteciales; la posición de los estomas; la ornamentación de los dientes del exostoma; la constitución y ornamentación de los segmentos del endostoma y el tamaño de las esporas.

6. *Orthotrichum scanicum* es una especie cuya distribución real abarca una gran parte del continente europeo y la Región Mediterránea, donde puede ser localmente abundante. Por este motivo debe excluirse de la Lista Roja Mundial de los briófitos amenazados según la IUCN.

7. Se excluye *Orthotrichum tenellum* de la flora de América. Se excluye *Orthotrichum consimile* de la flora de Europa.

8. *Orthotrichum columbicum* es la única de las especies tratadas en profundidad que presenta una disyunción transcontinental. El resto se restringen al occidente del Neártico (*Orthotrichum confusum, O. consimile, O. coulteri, O. cucullatum, O. franciscanum, O. norrisii, O. persimile* y *O. pilosissimum*) o del Paleártico (*Orthotrichum comosum, O. scanicum* y *O. tenellum*).

9. Todas las especies estudiadas en profundidad en esta memoria constituyen, de acuerdo con los marcadores empleados, grupos monofiléticos.

10. Tanto el supuesto complejo morfológico de *Orthotrichum consimile* (s.l.) como el de *Orthotrichum tenellum* (s.l.) están integrados en realidad por especies pertenecientes a linajes filogenéticos independientes y no constituyen grupos naturales. Atendiendo a su estructura filogenética existen indicios de procesos de especiación tanto simpátrica como alopátrica.

11. Las topologías resultantes del estudio de los marcadores cloroplásticos y nucleares son congruentes en cuanto a la delimitación específica, pero incongruentes en lo que respecta a las relaciones topológicas de varios grandes clados detectados en el seno de las especies de *Orthotrichum* con estomas inmersos.

12. Las dos aproximaciones empleadas para la delimitación específica de los supuestos complejos de *Orthotrichum consimile* (s.l.) y *Orthotrichum tenellum* (s.l.) han resultado compatibles en sus resultados y mutuamente imprescindibles para alcanzar una resolución sintética y robusta. De esta forma se pone de manifiesto la idoneidad de la taxonomía integrativa como marco metodológico para abordar el estudio de la biodiversidad en briófitos.

VII.

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“Porque, ¿cómo queréis vos que no me tenga confuso el qué dirá el antiguo legislador que llaman vulgo cuando vea que, al cabo de tantos años como ha que duermo en el silencio del olvido, salgo ahora, con todos mis años a cuestas, con una leyenda seca como un esparto, ajena de invención, menguada de estilo, pobre de conceptos y falta de toda erudición y doctrina; sin acotaciones en las márgenes y sin anotaciones en el fin del libro, como veo que están otros libros, aunque sean fabulosos y profanos, tan llenos de sentencias de Aristóteles, de Platón y de toda la caterva de filósofos, que admiran a los leyentes y tienen a sus autores por hombres leídos, eruditos y elocuentes?”

Miguel de Cervantes, 1605
Don Quijote de la Mancha. Prólogo de la primera parte.

A continuación se recopilan las referencias bibliográficas de las publicaciones citadas en los capítulos I, III y V. Cada uno de los artículos del capítulo IV cuenta con su propia sección bibliográfica.

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Taxón	Identif.	*atpB-rbcL*	*rps4*	*trnG*	*trnL*
Nyholmiella obtusifolia 1	O118	JQ836695	JQ836797	JQ836901	JQ8
Nyholmiella obtusifolia 2	O119	JQ836696	JQ836798	JQ836902	J
Ohotrichum alpestre 1	R669	JQ836760	JQ836864	JQ836967	
Ohotrichum alpestre 2	R670	JQ836761	JQ836865		
Ohotrichum alpestre 3	788PD	JQ836790	JQ836894		
Orthotrichum acuminatum	O102	JQ836693	JQ836795	JQ8	
Orthotrichum aequatoreum	1062PD	JQ836788	JQ836892		
Orthotrichum affine	O108	JQ836694	JQ836796		
Orthotrichum anomalum 1	562OR	JQ836784	JQ836888		
otrichum anomalum 2	787	JQ836785	JQ8369		
ichum anomalum 3	O120	JQ836697	JQ836		
chum bartramii 1	R598	JQ836734	JQ8		
m bartramii 2	R667	JQ836754			
artramii 3	R668	JQ83675			
cianum	R398	JQ83			
cum 1	R664	J			
2	R582				
	R599				
	R67				
	R				

nexo.

Relación de los números de registro de las secuencias en la base de datos de GenBank.

Taxón	Identif.	*atpB-rbcL*	*rps4*	*trnG*	*trnL-F*	*AdK*	*ort-Lfy*
Nyholmiella obtusifolia 1	O118	JQ836695	JQ836797	JQ836901	JQ836986	JQ837087	JQ837163
Nyholmiella obtusifolia 2	O119	JQ836696	JQ836798	JQ836902	JQ836987	JQ837088	JQ837164
Ohotrichum alpestre 1	R669	JQ836760	JQ836864	JQ836967	JQ837053	JQ837150	JQ837226
Ohotrichum alpestre 2	R670	JQ836761	JQ836865		JQ837054	JQ837151	JQ837227
Ohotrichum alpestre 3	788PD	JQ836790	JQ836894		JQ837083		
Orthotrichum acuminatum	O102	JQ836693	JQ836795	JQ836899	JQ836984		
Orthotrichum aequatoreum	1062PD	JQ836788	JQ836892		JQ837081		
Orthotrichum affine	O108	JQ836694	JQ836796	JQ836900	JQ836985		
Orthotrichum anomalum 1	562OR	JQ836784	JQ836888		JQ837077		
Orthotrichum anomalum 2	787	JQ836785	JQ836889		JQ837078		
Orthotrichum anomalum 3	O120	JQ836697	JQ836799	JQ836903	JQ836988	JQ837089	JQ837165
Orthotrichum bartramii 1	R598	JQ836734	JQ836838	JQ836942	JQ837027	JQ837124	JQ837200
Orthotrichum bartramii 2	R667	JQ836754	JQ836858	JQ836962	JQ837047	JQ837148	JQ837224
Orthotrichum bartramii 3	R668	JQ836755	JQ836859	JQ836963	JQ837048	JQ837149	JQ837225
Orthotrichum casasianum	R398	JQ836707	JQ836811	JQ836915	JQ837000	JQ837099	JQ837175
Orthotrichum columbicum 1	R664	JQ836762	JQ836866		JQ837055	JQ837145	JQ837221
Orthotrichum columbicum 2	R582	JQ836763	JQ836867	JQ836968	JQ837056	JQ837121	JQ837197
Orthotrichum columbicum 3	R599	JQ836764	JQ836868	JQ836969	JQ837057	JQ837125	JQ837201
Orthotrichum columbicum 4	R674	JQ836770	JQ836874		JQ837063	JQ837154	JQ837230
Orthotrichum columbicum 5	R676	JQ836772	JQ836876	JQ836976	JQ837065	JQ837156	JQ837232
Orthotrichum columbicum 6	R675	JQ836771	JQ836875	JQ836975	JQ837064	JQ837155	JQ837231
Orthotrichum columbicum 7	R678	JQ836773	JQ836877	JQ836977	JQ837066	JQ837158	JQ837234
Orthotrichum comosum 1	R652	JQ836745	JQ836849	JQ836953	JQ837038	JQ837137	JQ837213
Orthotrichum comosum 2	R653	JQ836746	JQ836850	JQ836954	JQ837039	JQ837138	JQ837214
Orthotrichum comosum 3	UAM2896	JQ836759	JQ836863	JQ836966	JQ837052	JQ837161	JQ837237
Orthotrichum comosum 4	R655	JQ836748	JQ836852	JQ836956	JQ837041	JQ837140	JQ837216
Orthotrichum comosum 5	R673	JQ836756	JQ836860	JQ836964	JQ837049	JQ837153	JQ837229

Taxón	Identif.	*atpB-rbcL*	*rps4*	*trnG*	*trnL-F*	*AdK*	*ort-Lfy*
Orthotrichum comosum 6	UAM2856	JQ836758	JQ836862		JQ837051	JQ837162	JQ837238
Orthotrichum confusum 1	R646	JQ836767	JQ836871	JQ836972	JQ837060	JQ837136	JQ837212
Orthotrichum confusum 2	R680	JQ836774	JQ836878	JQ836978	JQ837067	JQ837160	JQ837236
Orthotrichum consimile 1	R677	JQ836765	JQ836869	JQ836970	JQ837058	JQ837157	JQ837233
Orthotrichum consimile 2	R665	JQ836769	JQ836873	JQ836974	JQ837062	JQ837146	JQ837222
Orthotrichum consimile 3	R616	JQ836766	JQ836870	JQ836971	JQ837059	JQ837127	JQ837203
Orthotrichum consimile 4	R663	JQ836768	JQ836872	JQ836973	JQ837061	JQ837144	JQ837220
Orthotrichum coulteri 1	R311	JQ836705	JQ836808	JQ836912	JQ836997	JQ837097	JQ837173
Orthotrichum coulteri 2	R651	JQ836710	JQ836814	JQ836918	JQ837003	JQ837102	JQ837178
Orthotrichum coulteri 3	R564	JQ836713	JQ836817	JQ836921	JQ837006	JQ837105	JQ837181
Orthotrichum coulteri 4	R562	JQ836711	JQ836815	JQ836919	JQ837004	JQ837103	JQ837179
Orthotrichum coulteri 5	R563	JQ836712	JQ836816	JQ836920	JQ837005	JQ837104	JQ837180
Orthotrichum coulteri 6	R565	JQ836714	JQ836818	JQ836922	JQ837007	JQ837106	JQ837182
Orthotrichum coulteri 7	R613	JQ836735	JQ836839	JQ836943	JQ837028	JQ837126	JQ837202
Orthotrichum cucullatum 1	R574	JQ836723	JQ836827	JQ836931	JQ837016	JQ837113	JQ837189
Orthotrichum cucullatum 2	R578	JQ836727	JQ836831	JQ836935	JQ837020	JQ837117	JQ837193
Orthotrichum cucullatum 3	R575	JQ836724	JQ836828	JQ836932	JQ837017	JQ837114	JQ837190
Orthotrichum cucullatum 4	R623	JQ836737	JQ836841	JQ836945	JQ837030	JQ837129	JQ837205
Orthotrichum cucullatum 5	R577	JQ836726	JQ836830	JQ836934	JQ837019	JQ837116	JQ837192
Orthotrichum cucullatum 6	R579	JQ836728	JQ836832	JQ836936	JQ837021	JQ837118	JQ837194
Orthotrichum cucullatum 7	R576	JQ836725	JQ836829	JQ836933	JQ837018	JQ837115	JQ837191
Orthotrichum cucullatum 8	R620	JQ836736	JQ836840	JQ836944	JQ837029	JQ837128	JQ837204
Orthotrichum cucullatum 9	R657	JQ836750	JQ836854	JQ836958	JQ837043	JQ837141	JQ837217
Orthotrichum cucullatum 10	R658	JQ836751	JQ836855	JQ836959	JQ837044	JQ837142	JQ837218
Orthotrichum cupulatum 1	1105	JQ836782	JQ836886		JQ837075		
Orthotrichum cupulatum 2	1190	JQ836783	JQ836887		JQ837076		
Orthotrichum cupulatum 3	1108	JQ836789	JQ836893		JQ837082		

Taxón	Identif.	*atpB-rbcL*	*rps4*	*trnG*	*trnL-F*	*AdK*	*ort-Lfy*
Orthotrichum cupulatum 4	1036OR	JQ836786	JQ836890		JQ837079		
Orthotrichum cupulatum 5	1053	JQ836787	JQ836891		JQ837080		
Orthotrichum euryphyllum 1	1033	JQ836780	JQ836884		JQ837073		
Orthotrichum euryphyllum 2	1058PR	JQ836781	JQ836885		JQ837074		
Orthotrichum flowersii	R632	JQ836738	JQ836842	JQ836946	JQ837031		
Orthotrichum franciscanum 1	R569	JQ836718	JQ836822	JQ836926	JQ837011		
Orthotrichum franciscanum 2	R570	JQ836719	JQ836823	JQ836927	JQ837012	JQ837109	JQ837185
Orthotrichum franciscanum 3	R571	JQ836720	JQ836824	JQ836928	JQ837013	JQ837110	JQ837186
Orthotrichum franciscanum 4	R572	JQ836721	JQ836825	JQ836929	JQ837014	JQ837111	JQ837187
Orthotrichum franciscanum 5	R573	JQ836722	JQ836826	JQ836930	JQ837015	JQ837112	JQ837188
Orthotrichum franciscanum 6	R593	JQ836732	JQ836836	JQ836940	JQ837025	JQ837123	JQ837199
Orthotrichum franciscanum 7	R595	JQ836733	JQ836837	JQ836941	JQ837026		
Orthotrichum norrisii 1	R566	JQ836715	JQ836819	JQ836923	JQ837008		
Orthotrichum norrisii 2	R637	JQ836740	JQ836844	JQ836948	JQ837033	JQ837131	JQ837207
Orthotrichum norrisii 3	R567	JQ836716	JQ836820	JQ836924	JQ837009	JQ837107	JQ837183
Orthotrichum norrisii 4	R568	JQ836717	JQ836821	JQ836925	JQ837010	JQ837108	JQ837184
Orthotrichum norrisii 5	R659	JQ836752	JQ836856	JQ836960	JQ837045	JQ837143	JQ837219
Orthotrichum patens	1107PD	JQ836791	JQ836895		JQ837084		
Orthotrichum persimile 1	R580	JQ836729	JQ836833	JQ836937	JQ837022	JQ837119	JQ837195
Orthotrichum persimile 2	R666	JQ836753	JQ836857	JQ836961	JQ837046	JQ837147	JQ837223
Orthotrichum persimile 3	R581	JQ836730	JQ836834	JQ836938	JQ837023	JQ837120	JQ837196
Orthotrichum pilosissimum 1	R640	JQ836741	JQ836845	JQ836949	JQ837034	JQ837132	JQ837208
Orthotrichum pilosissimum 2	R642	JQ836742	JQ836846	JQ836950	JQ837035	JQ837133	JQ837209
Orthotrichum pilosissimum 3	R644	JQ836743	JQ836847	JQ836951	JQ837036	JQ837134	JQ837210
Orthotrichum pilosissimum 4	R645	JQ836744	JQ836848	JQ836952	JQ837037	JQ837135	JQ837211
Orthotrichum pulchellum 1	R681	JQ836775	JQ836879	JQ836979	JQ837068		
Orthotrichum pulchellum 2	R682	JQ836776	JQ836880	JQ836980	JQ837069		

Taxón	Identif.	*atpB-rbcL*	*rps4*	*trnG*	*trnL-F*	*AdK*	*ort-Lfy*
Orthotrichum pulchellum 3	R683	JQ836777	JQ836881	JQ836981	JQ837070		
Orthotrichum pulchellum 4	R684	JQ836778	JQ836882	JQ836982	JQ837071		
Orthotrichum pumilum	R152	JQ836700	JQ836802	JQ836906	JQ836991	JQ837092	JQ837168
Orthotrichum scanicum 1	R018	JQ836698	JQ836800	JQ836904	JQ836989	JQ837090	JQ837166
Orthotrichum scanicum 2	R037	JQ836699	JQ836801	JQ836905	JQ836990	JQ837091	JQ837167
Orthotrichum schimperi 1	R276	JQ836701	JQ836803	JQ836907	JQ836992		
Orthotrichum schimperi 2	R364	JQ836706	JQ836810	JQ836914	JQ836999	JQ837098	JQ837174
Orthotrichum schimperi 3	R362		JQ836809	JQ836913	JQ836998		
Orthotrichum schimperi 4	R656	JQ836749	JQ836853	JQ836957	JQ837042		
Orthotrichum schimperi 5	R634	JQ836739	JQ836843	JQ836947	JQ837032	JQ837130	JQ837206
Orthotrichum sharpii	R679	JQ836757	JQ836861	JQ836965	JQ837050	JQ837159	JQ837235
Orthotrichum stramineum 1	1109	JQ836792	JQ836896		JQ837085		
Orthotrichum stramineum 2	872	JQ83679	JQ836897		JQ837086		
Orthotrichum tenellum 1	R293	JQ836702	JQ836804	JQ836908	JQ836993	JQ837093	JQ837169
Orthotrichum tenellum 2	R294	JQ836703	JQ836805	JQ836909	JQ836994	JQ837094	JQ837170
Orthotrichum tenellum 3	R295	JQ836704	JQ836806	JQ836910	JQ836995	JQ837095	JQ837171
Orthotrichum tenellum 4	R296		JQ836807	JQ836911	JQ836996	JQ837096	JQ837172
Orthotrichum tenellum 5	R405	JQ836708	JQ836812	JQ836916	JQ837001	JQ837100	JQ837176
Orthotrichum tenellum 6	R409	JQ836709	JQ836813	JQ836917	JQ837002	JQ837101	JQ837177
Orthotrichum tenellum 7	R654	JQ836747	JQ836851	JQ836955	JQ837040	JQ837139	JQ837215
Orthotrichum tristriatum	1001PD	JQ836779	JQ836883		JQ837072		
Orthotrichum underwoodii	R583	JQ836731	JQ836835	JQ836939	JQ837024	JQ837122	JQ837198
Ulota crispa	O114	JQ836692	JQ836794	JQ836898	JQ836983		