VNiVERSiDAD D SALAMANCA
CAMPUS DE EXCELENCIA INTERNACIONAL

Facultad de Biología
Dpto. de Botánica y Fisiología Vegetal

Estudios biosistemáticos y filogeográficos en el género *Odontites* s.l. en el Mediterráneo Occidental y en la región Macaronésica

Tesis Doctoral

Daniel Pinto Carrasco

Salamanca, 2020

FACULTAD DE BIOLOGÍA

DEPARTAMENTO DE BOTÁNICA Y FISIOLOGÍA VEGETAL

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Memoria presentada por

Daniel Pinto Carrasco

para optar al Grado de Doctor por la

Universidad de Salamanca

VºBº del director

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D. **Enrique Rico Hernández** y Dña. **Mª Montserrat Martínez Ortega**, ambos Catedráticos de Botánica de la Universidad de Salamanca

AUTORIZAN, la presentación, para su lectura, de la Tesis Doctoral titulada **Estudios biosistemáticos y filogeográficos en el género *Odontites* s.l. en el Mediterráneo Occidental y en la región Macaronésica**, realizada por D. **Daniel Pinto Carrasco**, bajo su dirección, en la Universidad de Salamanca.

Y para que así conste a los efectos legales, expiden y firman el presente certificado en Salamanca, a 13 de Octubre de 2020.

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Fdo. Mª Montserrat Martínez Ortega

Común es el sol y el viento,
común ha de ser la tierra,
que vuelva común al pueblo
lo que del pueblo saliera.

—Luis López Álvarez*, Romance de los comuneros*—

"En España lo mejor es el pueblo. Siempre ha sido lo mismo. En los trances duros, los señoritos invocan la patria y la venden; el pueblo no la nombra siquiera, pero la compra con su sangre y la salva."

—Antonio Machado; Carta a Vigodsky, 20-02-1937—

V

Este mundo es el camino
para el otro, que es morada
sin pesar;
mas cumple tener buen tino
para andar esta jornada
sin errar.
Partimos cuando nacemos,
andamos mientras vivimos,
y llegamos
al tiempo que fenecemos;
así que cuando morimos,
descansamos.

XL

Así, con tal entender,
todos sentidos humanos
conservados,
cercado de su mujer
y de sus hijos y hermanos
y criados,
dio el alma a quien se la dio
(el cual la ponga en el cielo
en su gloria),
que aunque la vida perdió,
dejónos harto consuelo
su memoria.

Jorge Manrique, *Coplas a la muerte de su padre*—

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A todas las personas que han colaborado en la recolección de las muestras, tanto del grupo de investigación como externas al mismo, ya que sin su labor no se podrían haber hecho estos trabajos, ni los que se hagan en el futuro. Y espero que sean muchos, porque hay muestras para aburrir, de todo tipo y pelaje.

A Luís, por comenzar el trabajo con los *Odontites*, dejándose los ojos para contar los cromosomas; a Pablo por seguir con la morfometría del grupo de *O. vernus*, aunque esa tesina nunca viera la luz; y a Pascual, por conseguir sacar adelante su TFG pese a las dificultades.

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A todos los compañeros que han ido pasando por el Departamento de Botánica, ya sean estudiantes de TFG, TFM, doctorandos… que unos años más gente y otros menos, habéis hecho que en la sala de becarios siempre hubiese alguien con la que solucionar dudas (bastantes), comentar chorradas (más que bastantes) y compartir buenos ratos (más que más que bastantes). Y, como no puede ser de otra forma, cada uno de su padre y de su madre, y con la pedrada en un sitio diferente de la cabeza. Unos ya hace un tiempo que terminaron sus tesis/tesina/TFM (Bea, Santi, María, Sara, Blanca), otros hace menos que lo han hecho (Manu, David, Nélida, Bobo, Víctor, Walias, Paula, Noe), y finalmente me he quedado yo solo como guardián de la sala de becarios… hasta que llegue una nueva hornada de botánicos. Como dice la tonada "Ya se van los pastores a la Extremadura, ya se queda la sierra triste y oscura", pero espero que más pronto que tarde vuelva a haber caras nuevas por allí.

A los *Iter*, como ente con vida propia y con capacidad para perpetuarse pese a las adversidades, porque han hecho que estos últimos años hayan sido más amenos, y porque nos han demostrado que desde tener ínfulas de cirujanos estéticos sínicos hasta encontrar una especie nueva para la Península Ibérica pueden pasar solo unas pocas horas, y unos cuantos pacharanes. A Andrés, Faluke y Josefina, por los buenos ratos que nos hicieron pasar en Almería, y por hacernos ver que cualquier momento es bueno para coger un ramo de flores.

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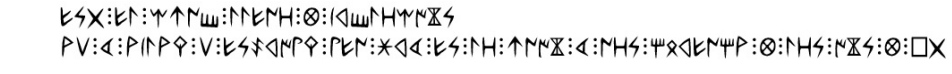
A todos los amigos del pueblo, J.F., Chaves, Miguelín, Emilio, Jabato, Mala Bestia, Rúben,

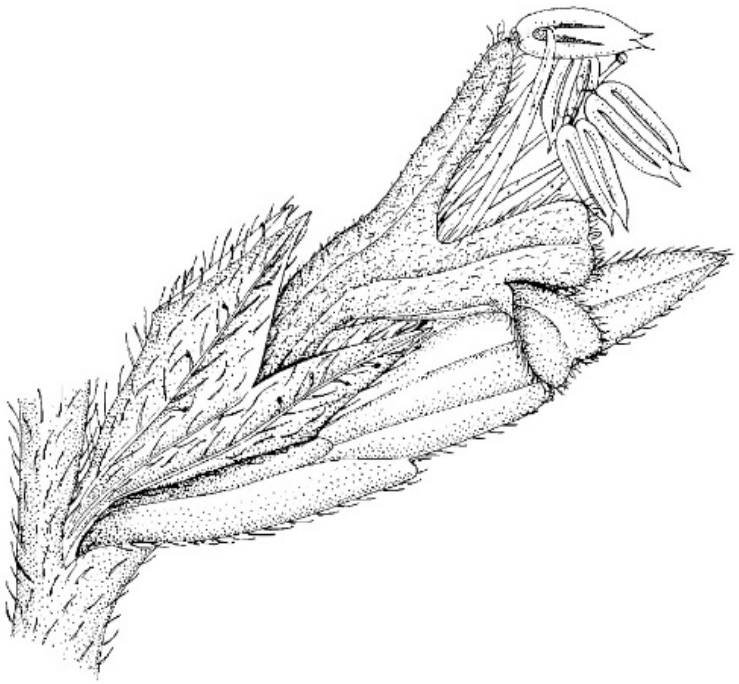
Carlos, Rodrigo, Diego, Victor, Mario, … que siempre estáis ahí para lo que haga falta. Echando la vista atrás, a los agradecimientos de la tesina, cómo ha cambiado la cosa. Ya no existe la mítica pampa, ni la fresita, ni el calibra, ni el corsita… la mitad estáis ya criando chiquillos, y/o construyendo casas. Y es que nos vamos haciendo mayores. La vida se abre camino.

Por último, quiero agradecer a mi familia todo lo que me han enseñado a lo largo de la vida. Parece que fue ayer, pero ya van 17 años viviendo lejos de ellos, y no hay día que, por H o por B, no me acuerde de alguno de vosotros. A mis hermanos, Ana, Mari, Vegui, Jose, Elena, y Bea, mis cuñados Javi y Álvaro, y a toda la recua de sobrinos, que cada vez sois más y más, Diego, Carlos, Marina, Cesar, Raúl, Gonzalo, Julia, y Laura. A mi madre, por todo lo que te preocupas siempre por mí, sobre todo cuando salgo al campo, y por aguantar mis rarezas. Y, muy especialmente, a mi padre, que nos dejó en 2018, pero sigue estando muy presente.

P.D.: estas dos frases por si a Josete (pese a no ser avéstico), o a algún otro “Indiana Jones”, le da por transcribirlo.



ÍNDICE DE CONTENIDOS



Capítulo 1:
Introducción general

ABSTRACT

This doctoral thesis expands on the studies on the genus *Odontites*, carried out by the Recognized Research Group (Grupo de Investigación Reconocido -GIR-) "Biodiversidad, sistemática y conservación de plantas vasculares y hongos (BIOCONS)", in the context of the macroproject *Flora iberica*. Molecular techniques and cytometry are used to complement the morphological and karyological knowledge already acquired. A chronological review is made of the most relevant publications on morphology and karyology of the genus, with special attention to the studies of our research group.

The content of each chapter of this doctoral thesis is hereby briefly described. It consists of 8 chapters, written in Spanish or English. The first chapter is an introduction, the next five (from 2 to 6) are scientific studies that can be read as if they were independent scientific papers (in fact, three of them have already been published in scientific journals), the seventh is a discussion, and the eighth includes the main conclusions of the entire doctoral thesis.

In addition, the main molecular phylogenies that concern the study group are reviewed in chronological order. Studies on the disintegration of the family Scrophulariaceae, on the recircumscription of the family Orobanchaceae and the tribes that comprise it, and on the phylogenetic relationships within the tribe Rhinantheae, are included. After that, the most interesting publications on the phylogenetic position of *Odontites* s.l., as well as its delimitation and related genera, are studied. And finally, the main objectives of this doctoral thesis are listed.
Keywords: *Odontites* and related genera; Orobanchaceae; phylogeny; Rhinantheae; taxonomic position.

RESUMEN

En esta Tesis Doctoral se continúan los estudios sobre el género *Odontites*, llevados a cabo por el Grupo de Investigación Reconocido (GIR) "Biodiversidad, sistemática y conservación de plantas vasculares y hongos (BIOCONS)", en el contexto del macroproyecto *Flora ibérica*. Se usan técnicas moleculares y citométricas para complementar el conocimiento morfológico y cariológico ya acumulado. Se hace un repaso cronológico de las publicaciones más relevantes sobre morfología y cariología del género, con especial atención a los estudios de nuestro grupo investigador.

Se describe someramente el contenido de cada capítulo de esta Memoria. Consta de 8 capítulos, escritos en castellano o en inglés. El primer capítulo es una introducción, los cinco siguientes (del 2 al 6) son estudios científicos que pueden leerse como si fuesen artículos científicos independientes (de hecho, tres de ellos ya han sido publicados en revistas

científicas), el séptimo es una discusión, y el octavo recoge las principales conclusiones de toda la Memoria.

Además, se revisan, en orden cronológico, las principales filogenias moleculares que conciernen al grupo de estudio. Se incluyen estudios sobre la desintegración de la familia Scrophulariaceae, sobre la recircunscripción de la familia Orobanchaceae y de las tribus que la integran, y sobre las relaciones filogenéticas dentro de la tribu Rhinantheae. A continuación, se estudian las publicaciones más interesantes sobre la posición filogenética de *Odontites* s.l., así como su delimitación y géneros afines. Y finalmente, se enumeran los objetivos principales de esta Tesis Doctoral.

Palabras clave: encuadre taxonómico; filogenia; *Odontites* y géneros afines; Orobanchaceae; Rhinantheae.

INTRODUCCIÓN GENERAL

Este trabajo surge como continuación de los estudios que el grupo de investigación "Biosistemática y biodiversidad de plantas vasculares", dirigido por el Dr. Enrique Rico Hernández, ha realizado sobre la familia Scrophulariaceae durante los últimos años en el contexto del macroproyecto *Flora iberica*. En 2015, el Grupo de Investigación Reconocido (GIR) "Biodiversidad, sistemática y conservación de plantas vasculares y hongos (BIOCONS)", dirigido por la Dra. M. Montserrat Martínez Ortega (del cual forma parte el doctorando que presenta esta memoria; http://biocons.usal.es/), tomó el relevo al anterior dando continuidad a las líneas de investigación ya abiertas e incorporando algunas nuevas. Con la Tesis Doctoral que nos ocupa, se pretendía ahondar en el conocimiento que ya se había generado al hacer la revisión taxonómica de los géneros *Odontites* Ludw., *Odontitella* Rothm. y *Macrosyringion* Rothm. para su publicación en *Flora iberica* (Rico, 2009). Esta vez se optó por el uso de técnicas moleculares (secuenciación de ADN y genotipado de individuos con diversos marcadores hipervariables) y citométricas que complementasen el conocimiento morfológico y cariológico ya acumulado, y se trató de que el estudio abarcara la totalidad del área de distribución del grupo de estudio, aunque se puso especial atención en el Mediterráneo Occidental (centro de diversidad del grupo). Así mismo, se pretendía aclarar las relaciones filogenéticas entre las especies y reconocer grupos naturales, lo cual permitiría finalmente realizar estudios detallados sobre los grupos de especies taxonómicamente más problemáticos.

Al comienzo de esta tesis nuestro grupo de trabajo ya tenía un conocimiento bastante profundo de la morfología de las especies presentes en la Península Ibérica, fruto de una revisión concienzuda de la bibliografía más relevante (principalmente los trabajos de M. Bolliger, W. Rothmaler y B. Snogerup -Rothmaler, 1943; Snogerup, 1977, 1982, 1983; Bolliger, 1985, 1993, 1996; Bolliger & al., 1990-), y del estudio del material depositado en los principales herbarios nacionales, así como de material relevante contenido en préstamos de varios herbarios europeos. Además, se buscó y recolectó material a lo largo y ancho de la Península Ibérica para tener una imagen más clara de la distribución y la variabilidad morfológica de cada especie. De esta manera, se pudo constatar, entre otras cosas, que algunas especies que habían sido citadas en la Península Ibérica, no se encuentran presentes en la misma, como es el caso de *Odonties purpureus* (Desf.) G. Don (confundido con *O. bolligeri* E. Rico, L. Delgado & Herrero, con *O. foliosus* Pérez Lara e incluso con *O. kaliformis* (Pourr. ex Willd.) Pau) y de *O. lanceolatus* (Gaudin) Reichenb. (en cuyo seno se subordinaba a *O. cebennensis* H.J. Coste & Soulié y a *O. pyrenaeus* (Bubani) Rothm.).

De forma paralela a los estudios morfológicos y corológicos, se indagó en la nomenclatura y se efectuaron tipificaciones de las especies objeto de estudio, detectando un buen número de sinónimos para las especies de más amplia distribución [*O. luteus* (L.) Clairv., *O. viscosus* (L.) Clairv.y *O. vernus* (Bellardi) Dumort.], sobre todo descritos por Carlos Pau y el Frère Sennen. Así, se neotipificó *Odontitella virgata* (Link) Rothm., se lectotipificó *Macrosyringion longiflorum* (Lam.) Rothm. (Rico & al., 2008b) y se aclaró la taxonomía del grupo de *O. purpureus*, describiendo una nueva especie (*O. bolligeri*) para sustituir el nombre inválido *O. squarrosus* subsp. *squarrosus* (Rico & al., 2008a).

Además, el Dr. Luís Delgado-Sánchez, como parte de su tesis doctoral, efectuó recuentos de cromosomas y estudió los cariotipos de la mayor parte de las especies y subespecies ibéricas de *Odontites* s.l. (*sensu lato*). Teniendo en cuenta, tanto estos recuentos de cromosomas, como los publicados anteriormente por otros autores, en la actualidad solo se desconoce el número de cromosomas de *O. pyrenaeus* subsp. *abilianus* P. Monts. y *O. cebennensis* (Delgado-Sánchez & al., 2015).

Finalmente, todo este conocimiento morfológico, corológico, cariológico y nomenclatural fue resumido y plasmado en los capítulos de *Flora iberica* dedicados a los géneros *Odontites*, *Odontitella* y *Macrosyringion* (Rico, 2009).

Esta tesis doctoral se ha realizado en gran parte en el marco del proyecto “Estudios filogenéticos y filogeográficos en el género *Odontites* Ludw. en el Mediterráneo Occidental”,

concedido por la Consejería de Educación de la Junta de Castilla y Léon (referencia SA143A08). Así mismo, los proyectos "*Flora iberica VIII* (Salamanca)", "*Flora iberica IX* (Salamanca)" y "Disentangling the intricate variation patterns in the diploid-polyploid complex *Veronica* subsect. *Pentasepalae* Benth. (*Veronica* L., Plantaginaceae sensu APG III). Relationships with the last glacial maximum (LGM) and the Mid-Holocene warm period" (referencias CGL2008-02982-C03-02/CLI, CGL2011-28613-C03-03 y CGL2012-32574, respectivamente) han contribuido a financiar este trabajo. Además, este trabajo también ha sido posible gracias al programa nacional FPU (Formación de Profesorado Universitario) del Ministerio de Ciencia e Innovación que concedió una beca-contrato al doctorando (referencia AP2008-03528) y financió dos estancias de investigación realizadas en la Carl-von-Ossietzky Universität Oldenburg – Alemania (años 2010 y 2012). Por otra parte, otra estancia realizada en la University of South Bohemia – República Checa (2013) fue costeada por los proyectos del grupo investigador.

ESTRUCTURA DE LA MEMORIA DE TESIS DOCTORAL

Con el fin de desarrollar los objetivos propuestos en la presente memoria doctoral, se ha estructurado la misma en 8 capítulos. Los capítulos 2-6 tienen formato de artículo científico y pueden leerse de forma independiente. Los capítulos 2-4 están escritos en inglés.

El **primer capítulo** es introductorio. En él se realiza el encuadre taxonómico de *Odontites* y géneros afines, y se ofrece una visión histórica del mismo. Además, se exponen de forma resumida los resultados de los escasos estudios previos de tipo filogenético disponibles para la totalidad de la tribu Rhinantheae y del género *Odontites*, lo que da una idea general del grado de conocimiento preexistente en el grupo. Por último, se indican los objetivos que se pretenden alcanzar durante el desarrollo de este trabajo.

En el **segundo capítulo** se presenta la más completa filogenia molecular hasta el momento de la tribu Rhinantheae, en la que se hace hincapié en el género *Odontites*. Se basa en secuencias de una región del ADN nuclear (ITS, *Internal Transcribed Spacer*) y dos de ADN plastidial (la región *trnK* y el intrón de *rps16*). Para la reconstrucción filogenética se han utilizado, tanto métodos de Inferencia Bayesiana, como de Máxima Parsimonia, así como algoritmos para generar redes filogenéticas. En este capítulo se pone en duda la actual delimitación de la tribu Rhinantheae, se recircunscribe el género *Odontites* de modo que se reincluye en él al género *Macrosyringion*, se analizan las relaciones filogenéticas entre los

taxones que integran el género, se discuten los caracteres morfológicos que apoyan los 5 grandes linajes encontrados, y se proponen 6 nuevas combinaciones nomenclaturales de acuerdo con nuestra hipótesis taxonómica. Este capítulo se ha publicado en la revista *Taxon* (Pinto-Carrasco & al., 2017).

En el **tercer capítulo** se hace un estudio filogeográfico y ecogenético del endemismo ibero-levantino *Odontites recordonii* Burnat & Barbey mediante el uso de marcadores genéticos hipervariables de tipo AFLP (*Amplified Fragment Length Polymorphism*). En este trabajo, se comparan los resultados obtenidos con diferentes métodos que permiten conocer la estructura genética de nuestras muestras (PCoA, NJ, análisis bayesiano de estructura genética poblacional…), y se realizan modelos de distribución potencial (tanto a tiempo actual, como proyecciones a tiempos pasados), para proponer una hipótesis filogeográfica y delimitar zonas refugio. Finalmente, se discuten las correlaciones existentes entre la presencia de ciertos alelos con las condiciones ambientales en las que viven los individuos que los portan, para intentar indagar en las presiones selectivas y/o evolutivas que han podido dar lugar a la estructura genética actual hallada. Este capítulo se encuentra en sus últimas fases de redacción y revisión antes de ser enviado para su publicación.

En el **cuarto capítulo** se desarrollan marcadores genéticos hipervariables de tipo microsatélite (o SSR, *Simple Sequence Repeat*) para *Odontites vernus* (Bellardi) Dumort., que es el taxón de mayor complejidad taxonómica del género. Se han obtenido un total de 14 parejas de cebadores útiles. Además, se evalúa la validez de 18 marcadores (incluidos los 14 anteriors) en otros 30 taxones cercanos (21 de *Odontites* y 9 de otros géneros de la tribu Rhinantheae). Este trabajo se ha publicado en la revista *Applications in Plant Sciences* (Pinto-Carrasco & al., 2016).

En el **quinto capítulo** se estudia la complejidad taxonómica y evolutiva de *Odontites vernus* en la Península Ibérica. Para ello se han utilizado los marcadores microsatélite desarrollados en el capítulo cuarto, así como secuencias de dos regiones del ADN plastidial (la región intergénica *trnK-rps16* y el intrón de *rps16*), y estimaciones del nivel de ploidía por medio de citometría de flujo. Se obtienen dos grupos de haplotipos plastidiales (herencia materna) bien diferenciados. Uno de ellos está integrado mayoritariamente por individuos diploides (más algunos tetraploides) y otro mayoritariamente por tetraploides (más unos pocos diploides). De ello se desprende que los individuos tetraploides ibéricos provienen de al menos

dos eventos de poliploidización independientes, probablemente por autopoliploidización. Los resultados de los SSRs (herencia biparental) muestran 4 grupos genéticos principales: dos de ellos integrados por individuos diploides que se corresponden completamente con los dos grupos de haplotipos cloroplásticos, y los otros dos por tetraploides donde se mezclan individuos de los dos grupos de haplotipos. Por lo tanto, es probable que los tetraploides, aún con orígenes diferentes, se puedan reproducir entre ellos, mientras que los diploides de orígenes diferentes parecen no hacerlo. Además, estos 4 grupos genéticos principales se pueden subdividir en 7 grupos con una distribución geográfica coherente. Este capítulo ha de ser traducido y revisado antes de ser enviado para su publicación.

El **sexto capítulo** recoge un escueto trabajo en el que se publica el redescubrimiento de *Odontites longiflorus* (sub. *Macrosyringion longiflorum*) en el Norte de África. Como resultado de los trabajos de campo realizados en Marruecos, se encontró esta especie tras casi un siglo desde que Mouret la recolectara en 1913. Este trabajo se ha publicado en *Acta Botanica Malacitana* (Pinto-Carrasco & al., 2011).

En el **séptimo capítulo**, para evitar repetir temas que ya se han discutido ampliamente en los diferentes capítulos de esta memoria, se recogen aquellos temas que aún no hayan sido tratados y, sobre todo, aquellos en los que, desde que se publicó el capítulo correspondiente en una revista científica, se ha publicado información relevante al respecto. Estos temas se discuten sobre la base de los resultados obtenidos en esta tesis doctoral.

Finalmente, en el **octavo capítulo,** se enumeran las principales conclusiones obtenidas en este estudio.

Cumpliendo con los requisitos para la obtención de la Mención de Doctor Internacional, cada capítulo de esta tesis incluye un resumen en castellano y otro en inglés, y las conclusiones están redactadas en ambos idiomas.

El abundante material vegetal recolectado para la realización de esta Tesis Doctoral está mayoritariamente depositado en el herbario de la Universidad de Salamanca (SALA), y puede consultarse electrónicamente a través de la *Global Biodiversity Information Facility* (GBIF), tanto en su nodo español (https://www.gbif.es/) como internacional (https://www.gbif.org/).

ENCUADRE Y ESTUDIOS TAXONÓMICOS PREVIOS DE *ODONTITES* Y GÉNEROS AFINES.

Reorganización y delimitación de la familia Scrophulariaceae s.l. con especial atención en las plantas parásitas

La delimitación de algunas de las familias de Angiospermas se ha visto ampliamente modificada en las últimas décadas debido a la creciente implantación de las técnicas de secuenciación de ADN y de inferencia filogenética (APG, 1998; APG II, 2003; APG III, 2009; APG IV, 2016). Uno de los casos más llamativos es el de la familia Scrophulariaceae. En su circunscripción tradicional (Wettstein, 1895), esta familia era la más extensa del orden Lamiales, y se distribuía por todo el planeta. En ella se incluían, a modo de "cajón de sastre", los géneros y especies que no reunían las características necesarias para reconocerlos como miembros de otras familias cercanas (Labiatae, Orobanchaceae, Verbenaceae, etc.). Los caracteres que tradicionalmente se mencionaban como típicos para esta familia son: flores de simetría bilateral, a menudo con corolas tubulares, ovarios con placentación axial, numerosos primordios seminales por carpelo, fruto capsular, y semillas con endospermo (Olmstead & al., 2001). Sin embargo, estos estados de carácter no son sinapomorfías morfológicas de esta familia ya que no son compartidos por todas las especies incluidas en la misma, y además algunos de ellos son compartidos con una o varias familias cercanas.

La ausencia de sinapomorfías morfológicas hacía suponer que las Scrophulariaceae, en su sentido tradicional, eran un grupo polifilético. Sin embargo, esto no se confirmó hasta que se estudiaron mediante secuencias de ADN plastidial de los genes *rbcL* y *ndhF*, detectándose dos clados en los que se reunían las muestras de Scrophulariaceae analizadas (Olmstead & Reeves, 1995). Otros estudios posteriores revalidaron estos resultados utilizando tanto secuencias de genes plastidiales como nucleares e incluso detectaron algunos linajes independientes más dentro de la familia (Young & al., 1999; Olmstead & al., 2001; Oxelman & al., 2005; Rahmanzadeh & al., 2005). Toda la información filogenética publicada hasta 2006 fue recogida por Tank y colaboradores en una revisión sistemática de las Scrophulariaceae s.l. (Tank & al., 2006). Posteriormente, se ha conseguido conocer las relaciones filogenéticas de algunos géneros controvertidos (Xia & al., 2009), así como el orden en el que divergieron las diferentes familias del orden Lamiales, donde se incluyen todas las disgregadas desde la familia Scrophulariaceae s.l. (Schäferhoff & al., 2010; Refulio-Rodriguez & Olmstead, 2014).

En medio de todos estos cambios taxonómicos recientes, todas las Scrophulariaceae parásitas (tanto holo- como hemiparásitas) han pasado a formar parte de las Orobanchaceae. E incluso las familias autótrofas Lindenbergiaceae (APG II, 2003) y Rehmanniaceae (APG IV,

2016) se consideran incluidas en este grupo.

En un estudio centrado en el origen del parasitismo, usando secuencias plastidiales (*rps2* y *matK*), se propuso por primera vez la inclusión de las tribus Rhinantheae y Buchnereae en las Orobanchaceae (Young & al., 1999), ya que junto a las Orobanchaceae tradicionales y *Lindenbergia* (anteriormente parte de la tribu Gratioleae) forman un grupo monofilético bien apoyado. Este resultado ha sido corroborado por filogenias posteriores, tanto basadas en ADN cloroplástico (*rbcL*, *ndhF* y *rps2*) (Olmstead & al., 2001), como en ADN nuclear (ITS) (Wolfe & al., 2005).

Entre los 32 géneros secuenciados en Wolfe & al. (2005) (el muestreo más extenso hasta ese momento), no se incluyó ninguna especie del género *Odontites sensu lato* (en adelante, *Odontites* s.l.). Sin embargo, las especies europeas de otros géneros quedaron reunidas en el mismo clado (al que denominaron "Bartsia clade"; Fig. 1A), a excepción de las pertenecientes a *Pedicularis* L. y *Orobanche* L. (y géneros afines). Por la semejanza morfológica y por una distribución geográfica común, se puede suponer que *Odontites* Ludw. está más relacionado con *Bartsia* L., *Parentucellia* Viv. y *Euphrasia* L., que con *Orobanche* o *Pedicularis*, y, por lo tanto, su pertenencia a ese clado europeo de las Orobanchaceae. En un estudio posterior, Bennett & Mathews (2006) incluyen una secuencia parcial del fitocromo A (*PHYA*) de una especie de *Odontites*, la cual se sitúa dentro del clado V (Fig. 1B), que es esencialmente el mismo que el "Bartsia clade" de Wolfe & al. (2005).

En todos los trabajos en los que el muestreo es suficientemente amplio, se observa que la tribu Rhinantheae en su circunscripción tradicional es polifilética (p.ej. Young & al., 1999; Olmstead & al., 2001; Wolfe & al., 2005). Los géneros americanos de las Rhinantheae (p.ej. *Castilleja* Mutis ex L.f., *Orthocarpus* Nutt., *Triphysaria* Fisch. & C.A.Mey., etc.) junto a *Pedicularis* (holártico) y algunos géneros americanos tradicionalmente incluidos en la tribu Buchnereae (p.ej. *Agalinis* Raf., *Seymeria* Pursh, etc.) forman un grupo monofilético bien diferenciado de los géneros mayoritariamente europeos (p.ej. *Bartsia* L., *Euphrasia* L., *Melampyrum* L., *Odontites* Ludw., *Rhinanthus* L., *Tozzia* L., etc.). Este último grupo es al que actualmente se llama tribu Rhinantheae. Si bien es cierto que algunos autores incluyen también algunos géneros asiáticos [*Pterygiella* Oliv. y géneros afines; (Dong & al. (2013); McNeal & al. (2013)] (Fig. 1C).

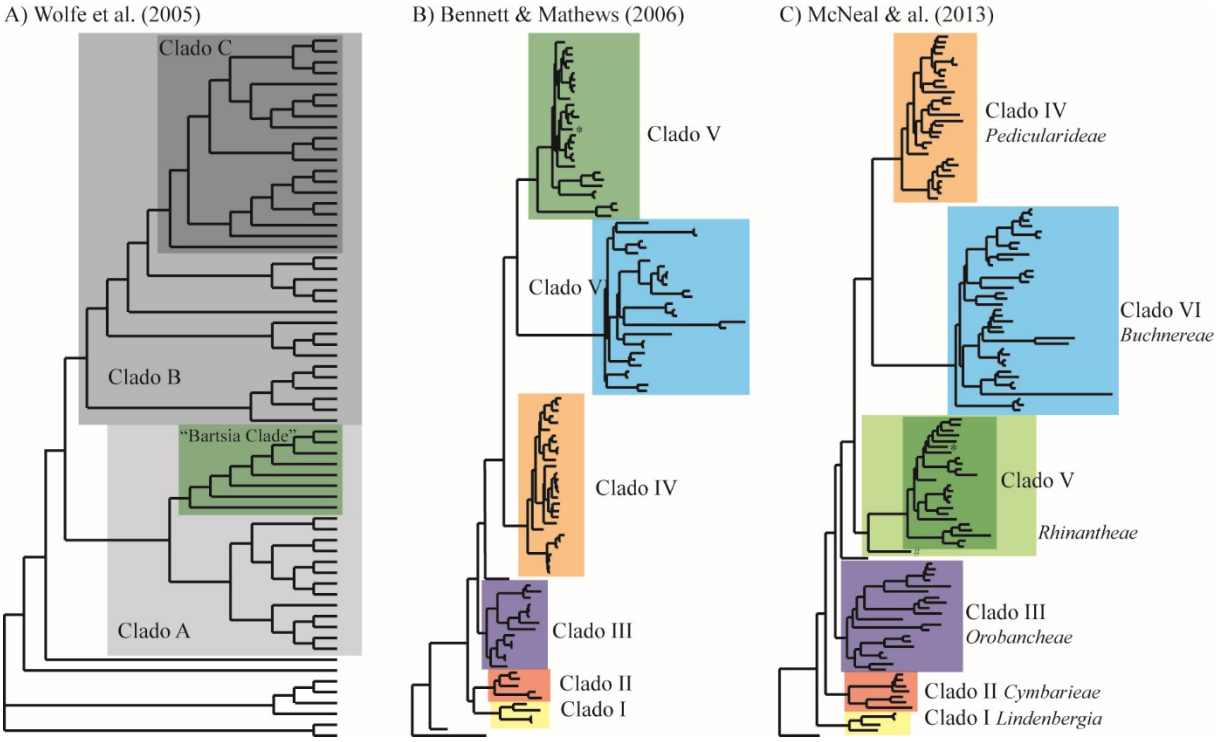


Figura 1. Resumen de los árboles filogenéticos publicados en los estudios más relevantes sobre la familia Orobanchaceae. Redibujado a partir de: A) Wolfe & al., (2005), B) Bennett & Mathews (2006) y C) McNeal & al. (2013). Las ramas terminales correspondientes a *Odontites* se han marcado con *. Nótese que, en McNeal & al. (2013), en la tribu Rhinantheae se incluye tanto el clado V como el género *Pterygiella* (marcado con #).

Posición filogenética y delimitación de *Odontites* y géneros afines.

Una vez conocemos la familia y tribu en la que se incluye nuestro grupo de estudio, debemos abordar otras cuestiones como las relaciones filogenéticas con otros géneros cercanos, la delimitación del propio género *Odontites*, así como las relaciones entre las especies que lo integran.

Odontites s.l. aglutina aproximadamente 31 especies y su distribución es mayoritariamente circunmediterránea (Bolliger, 1996). La mayor parte de las especies tienen áreas de distribución más o menos reducidas, mientras que solo tres de ellas tienen una distribución mucho más amplia. La zona de máxima diversificación para este género es el Mediterráneo Occidental, y se subdivide en dos áreas con alta endemicidad que son la Península Ibérica y el Norte de África (Marruecos, Argelia y Túnez) (Bolliger, 1993). Además, en el Mediterráneo Occidental están presentes 3 de los 4 géneros afines a *Odontites sensu stricto* (en adelante *Odontites* s.s.), lo cual nos indica que esta área ha tenido un papel muy relevante en la evolución de este grupo.

En las dos revisiones de *Odontites* más recientes (Rothmaler, 1943; Bolliger, 1996), se adopta un criterio restringido del género. Sin embargo, los caracteres utilizados en cada trabajo son diferentes: Rothmaler (1943) se basa sobre todo en la morfología de la corola, mientras que Bolliger (1996) le da mayor importancia a la ornamentación polínica (Bolliger & Wick, 1990). Siguiendo el criterio de Rothmaler (1943), *Odontites* s.l. se desintegra en *Macrosyringion* Rothm. (2 sp.), *Bornmuellerantha* Rothm. (2 sp.; Dönmez & Mutlu, 2010), *Odontitella* Rothm. (1 sp.) y *Odontites* s.s. (resto de especies). Además, Rothmaler (1943) propone la división de *Odontites* s.s. en 3 secciones (sect. *Dispermotheca* (Beauv.) Rothm., sect. *Orthantha* Benth. emend. Rothm. y sect. *Euodontites* Benth. emend. Rothm.). Estas secciones son claramente artificiales, puesto que reúnen especies morfológicamente muy diferentes (p.ej., *O. luteus* (L.) Clairv. y *O. kaliformis* (Willd.) Pau en la sect. *Orthantha*).

Por su parte, Bolliger (1996) revisa el género de forma mucho más extensa y minuciosa. Mantiene los mismos géneros que en la anterior revisión, pero además segrega un muevo género monoespecífico: *Bartsiella* Bolliger (1 sp.). Bolliger (1996) no propone subgéneros ni secciones dentro de *Odontites s.s.*, y se limita a reconocer algunos grupos de especies morfológicamente coherentes.

En el momento de comenzar y en las primaras fases del desarrollo de esta tesis doctoral, solo se había publicado un trabajo (Těšitel & al., 2010) en el que se incluyesen suficientes muestras de *Odontites* s.l. y otros géneros de la tribu Rhinantheae como para responder a las preguntas que se planteaban al comienzo de este apartado desde un punto de vista filogenético. De él podemos extraer las siguientes conclusiones: 1- los géneros más cercanos a *Odontites* son *Bartsia* s.l. (excepto *B. alpina* L.) y *Parentucellia;* 2- *Bornmuellerantha* debe reincluirse en *Odontites;* y 3- no es posible conocer las relaciones filogenéticas dentro de *Odontites* ya que solo se incluyen secuencias de 4 especies. Posteriormente, una nueva filogenia de la tribu (Scheunert & al., 2012) arrojó nueva luz sobre estas cuestiones. Las conclusiones más relevantes son: 1- los géneros más cercanos a *Odontites* serían *Bartsia* (en concreto las especies andinas), *Bellardia* y *Parentucellia* si atendemos al árbol construido con secuencias plastidiales, pero lo serían *Odontitella* y *Nothobartsia* si nos basamos en el inferido a partir de secuencias nucleares; 2- *Odontitella* claramente no forma parte de *Odontites*, hay que reintegrar a *Bartsiella* dentro de *Odontites*, y la posición de *Macrosyringion* es dudosa debido a la incongruencia entre los árboles generados con secuencias nucleares y plastidiales; y 3- se observan dos clados dentro de *Odontites* que morfológicamente son coherentes.

OBJETIVOS GENERALES

De acuerdo con el grado de conocimiento sobre *Odontites* y los géneros afines que existía en el momento de iniciar este trabajo, nos planteamos detectar patrones de estructuración genética en el seno del grupo, que puedan ser interpretados con criterios taxonómicos o geográficos. En este sentido, nos planteamos abordar los siguientes objetivos:

1. Comprobar si *Odontites* s.l. es monofilético (con especial interés en la posición del género *Macrosyringion*), investigar sus límites taxonómicos y, si fuera necesario recircunscribirlo, así como determinar su posición filogenética dentro de las Rhinantheae; todo ello a partir de un estudio filogenético con representación de todos los géneros de la tribu Rhinantheae.

2. Tratar de establecer qué relaciones filogenéticas existen entre los taxones que integran el género *Odontites* s.l., así como tratar de encontrar qué caracteres morfológicos pueden proporcionar apoyo a la hipótesis filogenética obtenida sobre la base de caracteres moleculares (secuencias de ADN). En relación con ello, proponer un nuevo tratamiento taxonómico para *Odontites* s.l., basado en el análisis filogenético de datos relativos a secuencias de ADN (regiones tanto nucleares como cloroplásticas) y en evidencias morfológicas y biogeográficas, así como proponer los cambios nomenclaturales que sean necesarios de acuerdo con el nuevo tratamiento taxonómico.

3. Estudiar el endemismo ibero-levantino *Odontites recordonii*, por medio de marcadores genéticos de tipo AFLP y modelos de distribución potencial, para conocer sus patrones filogeográficos, así como tratar de detectar señales de selección natural que pudieran estar correlacionadas con variables ecológicas relevantes. Asimismo, tratar de esclarecer, mediante evidencias genéticas, la delimitación de los taxones *O. kaliformis* s.s. y *O. recordonii*, que tradicionalmente han sido incluidos en *O. kaliformis* s.l.

4. Poner a punto las técnicas que permitan obtener marcadores moleculares de tipo SSR con niveles de variabilidad apropiados para resolver las diferentes cuestiones que pretendemos estudiar en *Odontites vernus* (especie utilizada en su desarrollo), así como valorar su transferibilidad a otros taxones tanto de *Odontites* como de la tribu Rhinantheae.

5. Llevar a cabo estudios en detalle sobre *Odontites vernus*, la especie más compleja del género, para conocer patrones genéticos y citotípicos intraespecíficos, que nos permitan establecer hipótesis sobre el origen del citotipo tetraploide, así como hipótesis filogeográficas para los diferentes grupos genéticos encontrados en la Península Ibérica.

6. Realizar recolecciones y observaciones en el campo que nos permitan aumentar el

conocimiento sobre la corología y hábitat de los taxones estudiados, y que nos ayuden a interpretar los patrones de diversificación, así como a profundizar en el conocimiento biosistemático.

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Capítulo 2:
Unravelling the phylogeny of the root-hemiparasitic genus *Odontites* (tribe Rhinantheae, Orobanchaceae): Evidence for five main lineages

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ABSTRACT

Despite the recent publication of several phylogenies focused on Rhinantheae, which has been expanded to include three Asian endemic genera, few studies so far have dealt with particular genera within the tribe. Here, we focus on *Odontites* and related genera because of the high morphological variability of the group and its unclear generic boundaries. Phylogenetic analyses were performed for nrDNA (ITS) and cpDNA (*trnK* region and *rps16* intron) datasets, using Bayesian and Parsimony analyses. Our results cast doubt on the inclusion of *Pterygiella* and related genera within the Rhinantheae and support the polyphyly of *Phtheirospermum*, making it necessary to propose three new combinations to avoid it. *Odontites* is recircumscribed to include *Bartsiella*, *Bornmuellerantha*, and *Macrosyringion*, but not *Odontitella*. Within *Odontites*, five distinct lineages are identified. These are distinguishable either by morphological synapomorphies or by a combination of several character states. Most of the *Odontites* species are regarded as monophyletic. In the *O. vernus* and *O. luteus* complexes, some taxonomic changes are made to avoid paraphyly, which results in three new combinations.

Keywords: *Odontites* and related genera; *Phtheirospermum*; phylogenetic incongruence; *Pterygiella* complex; Rhinantheae; species monophyly

RESUMEN

A pesar de la reciente publicación de varias filogenias centradas en la tribu Rhinantheae, la cual se ha ampliado para incluir tres géneros endémicos asiáticos, hasta ahora pocos estudios se han ocupado de géneros particulares dentro de la tribu. Aquí, nos centramos en *Odontites* y los géneros relacionados debido a la alta variabilidad morfológica del grupo y a sus límites genéricos poco claros. Se realizaron análisis filogenéticos para los sets de datos de DNA nuclear (ITS) y plastidial (región *trnK* e intrón de *rps16*), utilizando análisis bayesianos y de parsimonia. Nuestros resultados ponen en duda la inclusión de *Pterygiella* y géneros afines dentro de la tribu Rhinantheae y apoyan la polifilia de *Phtheirospermum*, por lo que es necesario proponer tres nuevas combinaciones para evitarlo. *Odontites* se recircunscribe para incluir *Bartsiella*, *Bornmuellerantha* y *Macrosyringion*, pero no *Odontitella*. Dentro de *Odontites*, se identifican cinco linajes distintos. Estos se distinguen por sinapomorfías morfológicas o por una combinación de varios estados de carácter. La mayoría de las especies de *Odontites* se consideran monofiléticas. En los complejos de *O. vernus* y *O. luteus*, se realizan algunos cambios taxonómicos para evitar la parafilia, lo que da como resultado tres nuevas combinaciones.

Palabras clave: Complejo de *Pterygiella*; incongruencia filogenética; monofilia de las especies; *Odontites* y géneros relacionados; *Phtheirospermum*; Rhinantheae.

INTRODUCTION

Traditionally, all root-parasitic Scrophulariaceae (today considered part of Orobanchaceae; Young & al., 1999; Bennett & Mathews, 2006; McNeal & al., 2013) have been included in tribe Rhinantheae. The data available to date show that this tribe is not monophyletic. However, a subgroup of 15–20 genera (depending on different taxonomic treatments) forms a natural group, which includes the type genus *Rhinanthus* L. ("Bartsia clade" in Wolfe & al., 2005, or "Clade V" in Bennett & Mathews, 2006 and McNeal & al., 2013). The tribe Rhinantheae has recently been redefined as the "least inclusive crown clade that includes: *Pterygiella nigrescens* Oliv. 1896, *Rhinanthus crista-galli* L. 1753, *Melampyrum pratense* L. 1753, and *Tozzia alpina* L. 1753" (McNeal & al., 2013). This tribe has been the focus of four phylogenetic studies. The first two (Těšitel & al., 2010; Scheunert & al., 2012) improved the taxon sampling and filled sampling gaps. They corroborated the monophyly of the tribe, identified major lineages within it, and proposed some taxonomic changes including new nomenclatural combinations. Later on, Uribe-Convers & Tank (2015) and Gaudeul & al. (2016) investigated the diversification dynamics and biogeography of *Bellardia* All. s.l. (including *Bellardia*, *Parentucellia* Viv., and *Neobartsia* Uribe-Convers & Tank; "Bellardia clade" in Scheunert & al., 2012) and *Odontites* Ludw., respectively, and made use of the phylogeny of Rhinantheae only as an evolutionary framework. None of these studies included samples of the endemic Chinese genus *Pterygiella* Oliv. or of the related genera *Phtheirospermum* Bunge ex Fisch. & C.A.Mey., *Xizangia* D.Y.Hong and *Pseudobartsia* D.Y.Hong. Apart from investigations at tribal level, there are few molecular studies on particular genera of the Rhinantheae (*Euphrasia* L., Gussarova & al., 2008; the "*Pterygiella* complex", Dong & al., 2013; *Neobartsia*, Uribe-Convers & Tank, 2015; Uribe-Convers & al., 2016).

Figure 1 summarizes the phylogenetic hypotheses of the "core group of Rhinantheae" (sensu Scheunert & al., 2012) proposed so far (Těšitel & al., 2010; Scheunert & al., 2012; McNeal & al., 2013; Uribe-Convers & Tank, 2015; Gaudeul & al., 2016). In most of them, *Odontites* (including *Bornmuellerantha* Rothm.) was deemed sister to the Bellardia clade in cpDNA and combined datasets (nrDNA+cpDNA). Only Scheunert & al. (2012) and Gaudeul & al. (2016) included samples of *Bartsiella* Bolliger, *Macrosyringion* Rothm. and *Odontitella* Rothm. in their studies. Scheunert & al. (2012) found *Odontitella* to be sister to *Nothobartsia* Bolliger & Molau, supporting its segregation from *Odontites*. In Scheunert & al. (2012) and Gaudeul & al. (2016),

Bartsiella and *Bornmuellerantha* were nested within *Odontites* s.str. (sensu Bolliger, 1996), and both genera were consequently re-included in it. Following Scheunert & al. (2012), the position of *Macrosyringion* remained doubtful due to topological incongruence and low clade support (in the cpDNA tree). Gaudeul & al. (2016) did not detect the same relationships at the genus level as previous authors did, probably due to limited taxon sampling (*Nothobartsia*, *Neobartsia*, and *Hedbergia* Molau were not included). This latter study found *Macrosyringion* as sister to the clade containing *Bellardia* and *Parentucellia* with high support.

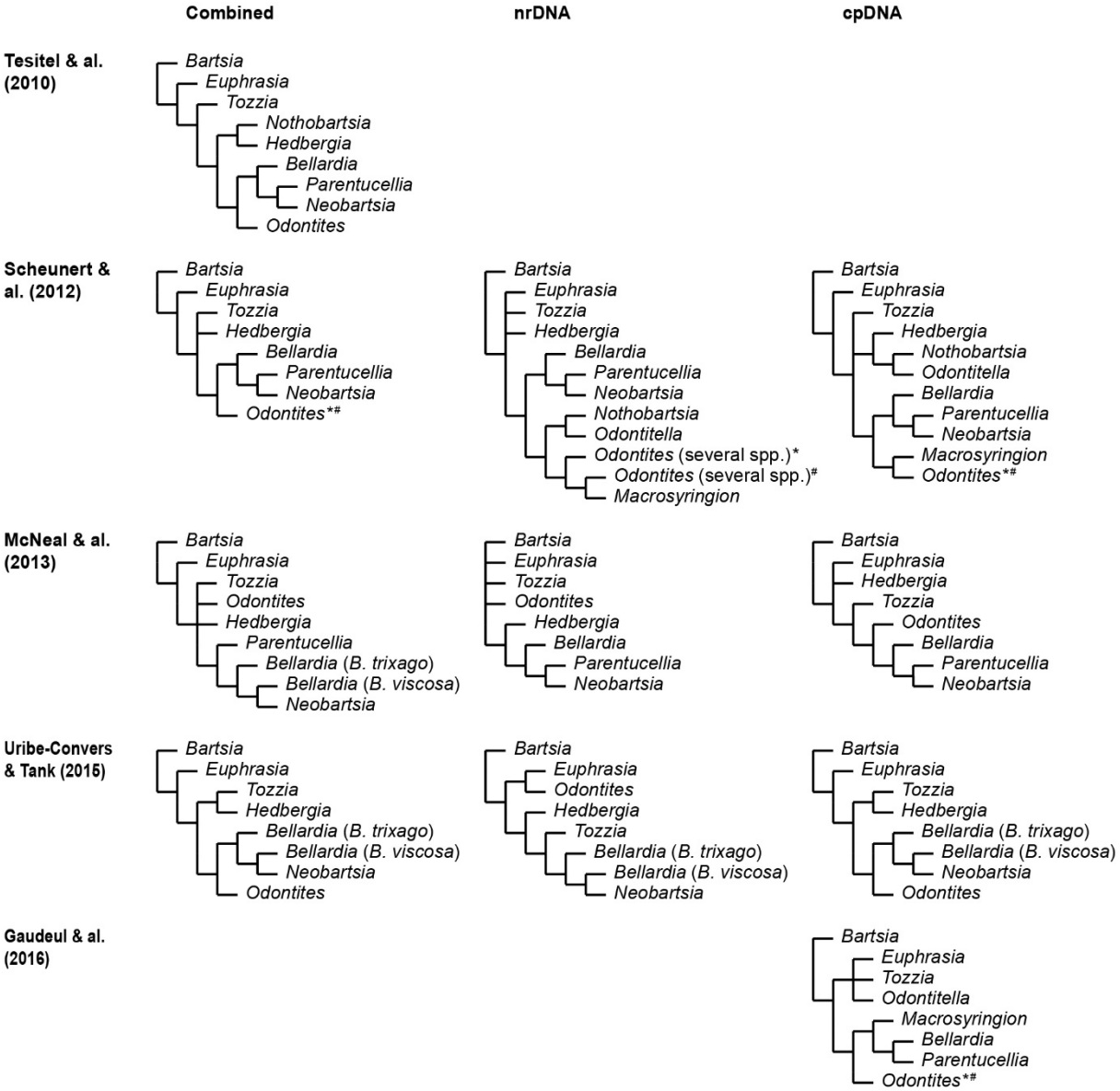

Fig. 1. Summary of the tree topologies obtained from the nrDNA, cpDNA and combined datasets published in the most relevant studies of tribe Rhinantheae (redrawn from Těšitel & al., 2010; Scheunert & al., 2012; McNeal & al., 2013; Uribe-Convers & Tank, 2015; and Gaudeul & al., 2016). Trees were pruned to only show the "core group of Rhinantheae" (sensu Scheunert & al., 2012), and branches with low support were collapsed into polytomies. Clades containing *Bartsiella* and *Bornmuellerantha* samples were marked with * and # respectively.

Odontites (with ca. 30 species and 15 subspecies in its traditional and broadest sense, hereafter referred to as *Odontites* s.l.; Bolliger, 1993) is distributed throughout temperate Eurasia, the Mediterranean region (including several islands), and Macaronesia, with a diversification centre in the WesternMediterranean area. Its taxonomy has long been controversial. Linnaeus (1753) described several species of *Odontites* under *Euphrasia*, and later taxonomists added newly discovered species and proposed several rearrangements (e.g., Don, 1838; Kerner, 1888; Beauverd, 1911). Most recently, Rothmaler (1943) segregated *Bornmuellerantha*, *Macrosyringion*, and *Odontitella* from *Odontites* s.l. The remaining species were divided into three sections based on the morphology of the corolla and stamens (sect. *Dispermotheca* (Beauv.) Rothm., sect. *Orthantha* Benth. em. Rothm., sect. *Euodontites* Benth. em. Rothm.). Although this proposal was not considered by Webb & Camarasa (1972), Bolliger (1996) recognized the three small genera segregated by Rothmaler (1943), and even separated an additional genus, *Bartsiella*, based on pollen exine sculpturing and calycinal glandular hairs (Bolliger, 1985; Bolliger & Wick, 1990). Bolliger (1996) included 26 species in *Odontites* s.str. and did not accept sections within the genus, although several species groups were recognized. Most are present in the Iberian Peninsula (Rico, 2009), and recently this area has been postulated as the centre of origin of a clade composed of *Odontites* s.str., *Bartsiella*, and *Bornmuellerantha* (Gaudeul & al., 2016).

Morphologically, *Odontites* s.l. has character states which are intermediate between those of *Euphrasia* and *Bartsia* L. (Bolliger, 1996). Marked morphological variability exists within *Odontites* s.l., especially regarding corolla shape and colour, as well as calyx and corolla indument (Fig. 2). Many vegetative and reproductive characters appear to have undergone parallel, convergent, and reverse evolution (Bolliger, 1993). Furthermore, some morphological characters and phenology are influenced by host plants (Snogerup, 1982), and in some species groups seasonal ecotypes exist (ter Borg, 1985; Bolliger, 1996; Koutecký & al., 2012). Therefore, the delimitation of species and the analysis of species relationships are difficult when based only on morphology.

Hybridization and incomplete lineage sorting (ILS) are the most widely studied processes that might explain gene-tree incongruence (Kubatko, 2009). Hybridization events can also result in allopolyploid speciation; recurrent formation of polyploids in plants has been shown to be the rule rather than the exception (Soltis & Soltis, 1993, 1999). However, other biological processes (e.g., gene duplication and horizontal gene transfer) may also be a source of phylogenetic incongruence. Regarding the members of tribe Rhinantheae, several cases of incongruence between gene trees involving several genera, (e.g., the clade *Odontitella*+*Nothobartsia*; Scheunert

& al., 2012), individual genera (e.g., *Tozzia* L. or *Odontites*; Uribe-Convers & Tank, 2015) and species within genera (e.g., *Bartsia* sp.; Scheunert & al., 2012) have been detected. Polyploidization is not extensive within *Odontites* s.l., although two taxa, *O. jaubertianus* (Boreau) D.Dietr. and *O. vernus* (Bellardi) Dumort. subsp. *vernus*, could be the result of allo- and autopolyploidization, respectively (Bolliger & al., 1990; Bolliger, 1996).

The aims of this study were to: (1) test the monophyly and topological position of *Odontites*, using a complete phylogenetic framework (i.e., all genera included in tribe Rhinantheae sensu McNeal & al., 2013); (2) determine the usefulness of several morphological characters considered relevant in previous taxonomic treatments in delimiting and supporting monophyletic groups, especially in *Odontites* and related genera; and (3) finally, test the monophyly of those species in the Odontites clade for which more than one specimen was collected, covering the entire species distribution range.

MATERIALS AND METHODS

Plant material. — Taxon sampling covered a representative number of species of all genera included in tribe Rhinantheae (clade V + *Pterygiella* complex; McNeal & al., 2013). A total of 350 ingroup individuals (747 newly generated sequences) were included in the analyses. They represent 86 species of 17 genera. For correct choice of outgroup taxa, the most comprehensive molecular phylogeny of Orobanchaceae to date (McNeal & al., 2013) was followed. One representative species of tribe Buchnereae Benth. (*Striga asiatica* (L.) Kuntze; clade VI) together with three of tribe Pedicularideae Duby (*Seymeria laciniata* (M.Martens & Galeotti) Standl., *Pedicularis groenlandica* Retz. and *Phtheirospermum japonicum* (Thunb.) Kanitz; clade IV) were chosen to root the phylogenetic analyses. All sequences used in the study (from GenBank and newly generated data with voucher information) are listed in Appendix 1. The keys provided by Bolliger (1996) and Rico (2009) were used to identify most of the herbarium vouchers used here. Seven species belonging to *Odontites* s.str., which are narrowly endemic in North Africa (Algeria and Tunisia; 5 spp.), Sicily (1 sp.), and the Near East (Lebanon and Syria; 1 sp.), were not included in this study because it was not possible to obtain good-quality DNA samples.

DNA extraction and amplification. — Total genomic DNA was isolated either from silica-gel-dried tissue (leaves and/or bracts) or from herbarium samples using the modified CTAB method (Doyle & Doyle, 1987) or the NucleoSpin Plant Kit following the protocols of the manufacturer (Macherey-Nagel, Düren, Germany). DNA was diluted in 50 µl of buffer. Concentration and quality were assessed by spectrophotometry and electrophoresis (1.0%

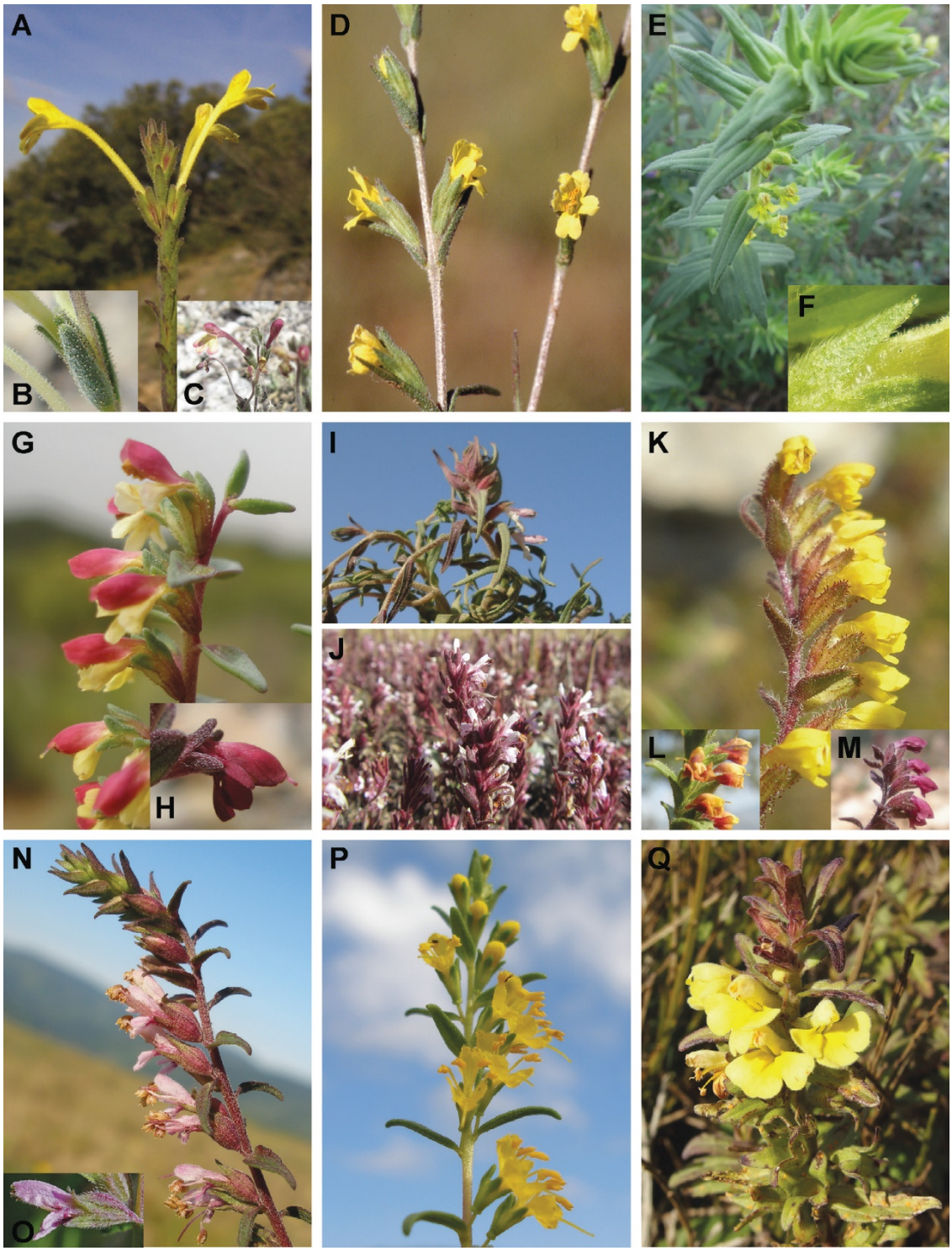

Fig. 2. Morphological diversity in *Odontites*. Herbarium number or locality are indicated in parentheses. **A–C,** Macrosyringion lineage; **D,** Bornmuellerantha lineage; **E & F,** Pyrenaeus lineage; **G–M,** Viscosus lineage; **N–Q,** Vernus lineage. **A,** *O. longiflorum* (Spain, Burgos, Montes Obarenes); **B,** *O. longiflorum* (SALA 135639; detail of calyx and corolla indument); **C,** *O. longiflorum* (SALA 110071; variability in corolla colour); **D,** *O. aucheri* (SALA 120807); **E,** *O. cebennensis* (SALA 156186); **F,** *O. cebennensis* (SALA 135679; detail of calyx and corolla indument); **G,** *O. foliosus* (SALA 134537); **H,** *O. bolligeri* (SALA 142142, detail showing a glabrous corolla); **I,** *O. rameauanus* (MA 746138); **J,** *O. maroccanus* (SALA 156178); **K,** *O. viscosus* (SALA 137360); **L & M,** *O. viscosus* (SALA 110047 and SALA 137373; variability in corolla colour); **N,** *O. vernus* (SALA 135643); **O,** *O. vernus* (Spain, Valladolid, San Miguel del Arroyo; detail of calyx and corolla indument); **P,** *O. luteus* (SALA 135662); **Q,** *O. hollianus* (MA 714540). — Photographs by S. Andrés-Sánchez (A, H, L), D. Pinto-Carrasco (B, C, E, J), E. Rico (D, F, G, I, K, M, N, P, Q) and J. Bobo-Pinilla (O).

agarose gel), respectively. One nuclear ribosomal region (including the ITS1 and ITS2 spacer regions and the 5.8S rRNA gene) plus two non-coding chloroplast regions (part of the *trnK* region and the *rps16* intron) were chosen for phylogenetic analyses. Reagent concentrations, PCR profiles, and primers followed Scheunert & al. (2012). Most of the markers were sequenced bidirectionally with the same primer pairs as used for amplification, using BigDye chemistry on an ABI 3730XL analyzer at Macrogen Europe or at Ludwig-Maximilians-University sequencing services.

Sequence editing, alignment, and indel-coding. — All newly generated sequences were edited, assembled, and automatically aligned using Geneious v.5.5.8 (Kearse & al., 2012). Online available sequences completing taxon sampling were taken mainly from Těšitel & al. (2010), Scheunert & al. (2012), Dong & al. (2013) and Uribe-Convers & Tank (2015). They were trimmed to fit the length of the newly generated sequences and added to the alignments. Some minor adjustments were made manually after visual inspection, and mononucleotide repeats (≥5 bp) were excluded from further analysis. Insertions and deletions (indels) were coded according to the simple indel-coding method (Simmons & Ochoterena, 2000), as implemented in SeqState v.1.4.1 (Müller, 2005). They were added to the data as a binary matrix and, consequently, gaps were treated as missing data.

Datasets and phylogenetic analyses. — Two datasets were analysed independently: (1) nrDNA (ITS) and (2) cpDNA (*trnK*+*rps16*). Since both soft and hard (posterior probabilities [PP] ≥0.80 and ≥0.95 respectively) topological incongruences between nrDNA and cpDNA trees were detected (see the paragraph "Visualization of topological incongruence" below), analyses using a combined dataset were not performed.

Phylogenetic analyses were conducted using both Bayesian inference (BI) and maximum parsimony (MP). Bayesian analyses were performed with MrBayes v.3.2 for 64-bit systems (Ronquist & al., 2012), using the best substitution model for each sequenced region identified using the BIC criterion as implemented in jModeltest v.2.1.4 (Darriba & al., 2012). The indel partitions were treated as restriction data and analysed using the model settings recommended by Ronquist & al. (2009). Short preliminary runs were carried out with different hot chain temperatures (in the range 0.01–0.2) to check for swapping efficiency among chains. For the final analyses, the temperature parameter was fixed to temp = 0.025. Two Markov chain Monte Carlo (MCMC) runs with four chains each (one cold chain, three hot chains) were started from independent random seeds and computed 10 million generations, with trees sampled every 1000th generation. After discarding a burn-in of 3000 trees (30% of all sampled trees) from each run, a majority-rule consensus tree was calculated. Traces were visually inspected in Tracer v.1.6

(Rambaut & al., 2015) to ensure that the effective sample sizes (ESSs) of all parameters were >200, as recommended by the authors, and to check the convergence of parameter estimates across runs. Nodes with PP ≥ 0.95 were considered to be strongly supported (Huelsenbeck & Rannala, 2004).

Parsimony analyses were conducted with TNT (Tree analysis using New Technology) v.1.1. (Goloboff & al., 2008), applying the traditional search option (TBR, Tree Bisection-Reconnection branch swapping) with equal character weights. In an initial run, 10,000 random addition sequence replicates were performed, using TBR branch-swapping and saving 10 trees per replicate. Since some replicates reached the maximum number of saveable trees, the trees from the first run were used as starting trees in a second heuristic search. Bootstrap support (BS) was calculated with 1000 replicates, each consisting of 500 random addition sequence replicates using TBR branch-swapping (saving 100 trees per replicate) in PAUP* v.4.0b10 (Swofford, 2002). BS values ≥70 were considered to indicate good node support (Hills & Bull, 1993). Consistency index (CI), retention index (RI), and rescaled consistency index (RC) were likewise calculated using PAUP*.

Visualization of topological incongruence. — To represent the differences between trees obtained with differently inherited markers (biparental vs. maternal, i.e., ITS vs. cpDNA) as a network, network building algorithms were run using the corresponding consensus trees. As a means of maintaining only statistically robust nodes, those with PP < 0.80 in majority-rule consensus trees from the Bayesian analysis were collapsed using Mesquite (Maddison & Maddison, 2014). Using this relatively low threshold, soft and hard incongruences can be shown at the same time. Collapsed trees were imported into SplitsTree v.4 (Huson & Bryant, 2006). The SuperNetwork algorithm was run with the Edge Weights option set to none (branch lengths are not taken into account). The network was not rooted in order to avoid graphical distortion.

RESULTS

Sequencing and alignments. — For this study, a total of 747 sequences was generated: 248 for ITS, 249 for the *rps16* intron, and 250 for the *trnK* region. As most of the ITS sequences provided unambiguous pherograms (i.e., without any signs of length polymorphisms), no cloning was performed, and, when required, single nucleotide polymorphisms were coded as IUPAC Nucleotide Codes. In addition to the newly generated sequences, 247 sequences were taken from GenBank (http://www.ncbi.nlm.nih.gov) to complete the taxon sampling. Table 1 summarizes details of alignment statistics for all markers and datasets including the proportions

of parsimony-informative characters and the models of molecular evolution.

The topology of the 50% majority-rule consensus trees from the maximum parsimony and Bayesian analyses proved almost identical and differed only in the topology of some nodes that were poorly supported. As Bayesian trees were generally better resolved and supported, only the Bayesian topologies are shown. Bootstrap support derived from MP analyses were added to these trees.

Table 1. Summary of sequence characteristics and analysis results for the different regions and datasets.

	ITS	*rps16*	*trnK*	cpDNA
Number of individuals	353	308	333	349
Taxon sampling: species (genera)	87 (21)	65 (19)	75 (20)	83 (20)
Length of newly generated sequences in bp (average)	577–734 (695)	524–861 (826)	676–1057 (1038)	–
Aligned length in bp, after trimming	797	952	1128	2080
Number of coded indels	138	85	64	149
% total missing data	6.3	3.3	3.0	10.7
Model of molecular evolution (Bayesian information criterion)	SYM+I+G	TVM+G	TVM+G	–
No. of variable sites (%)	579 (61.9)	–	–	949 (42.6)
No. of parsimony-informative sites (%)	483 (51.7)	–	–	621 (27.9)
Tree length of most parsimonious trees	2123	–	–	1614
Consistency index (CI)	0.4207	–	–	0.7127
CI excluding uninformative sites	0.3931	–	–	0.6347
Retention index (RI)	0.9203	–	–	0.9491
Rescaled consistency index (RC)	0.3872	–	–	0.6764

Major clades in the ITS and cpDNA trees. — The topology of the cpDNA tree was generally similar to that of the ITS tree (Fig. 3; Electr. Suppl.: Fig. S1), but support values were overall slightly lower in the cpDNA tree. All genera were recovered as well supported clades (in one or both trees), with the exception of *Phtheirospermum.* The generitype species, *Ph. japonicum,* grouped within tribe Pedicularideae (sensu McNeal & al., 2013; clade A; ITS PP = 1; BS = 97, cpDNA PP = 1; BS = 100; Electr. Suppl.: Fig. S1), while the remaining three species formed a clade with *Pterygiella* (ITS PP = 1; BS = 100, cpDNA PP = 1; BS = 86).

Comparing ITS and cpDNA topologies (Fig. 3; Electr. Suppl.: Fig. S1), only two cases (affecting six genera) of phylogenetic incongruence at generic or higher taxonomic levels were detected. These are visualized in a SuperNetwork (see below, Fig. 6A). The first is clade B (ITS PP = 0.93; BS = 86, cpDNA PP = 1; BS = 51; "Pterygiella complex II" sensu Dong & al., 2013; i.e., a clade composed of *Pterygiella*, *Pseudobartsia* and *Xizangia*) that was sister to tribe Rhinantheae s.str. (clades C–K) in cpDNA but related to tribe Pedicularideae (clade A) in ITS. The second concerns *Euphrasia* (clade H) and a clade that includes *Odontitella* and *Nothobartsia* (clade J). *Euphrasia* was part of a clade containing *Hedbergia* and *Tozzia* in ITS, but it was

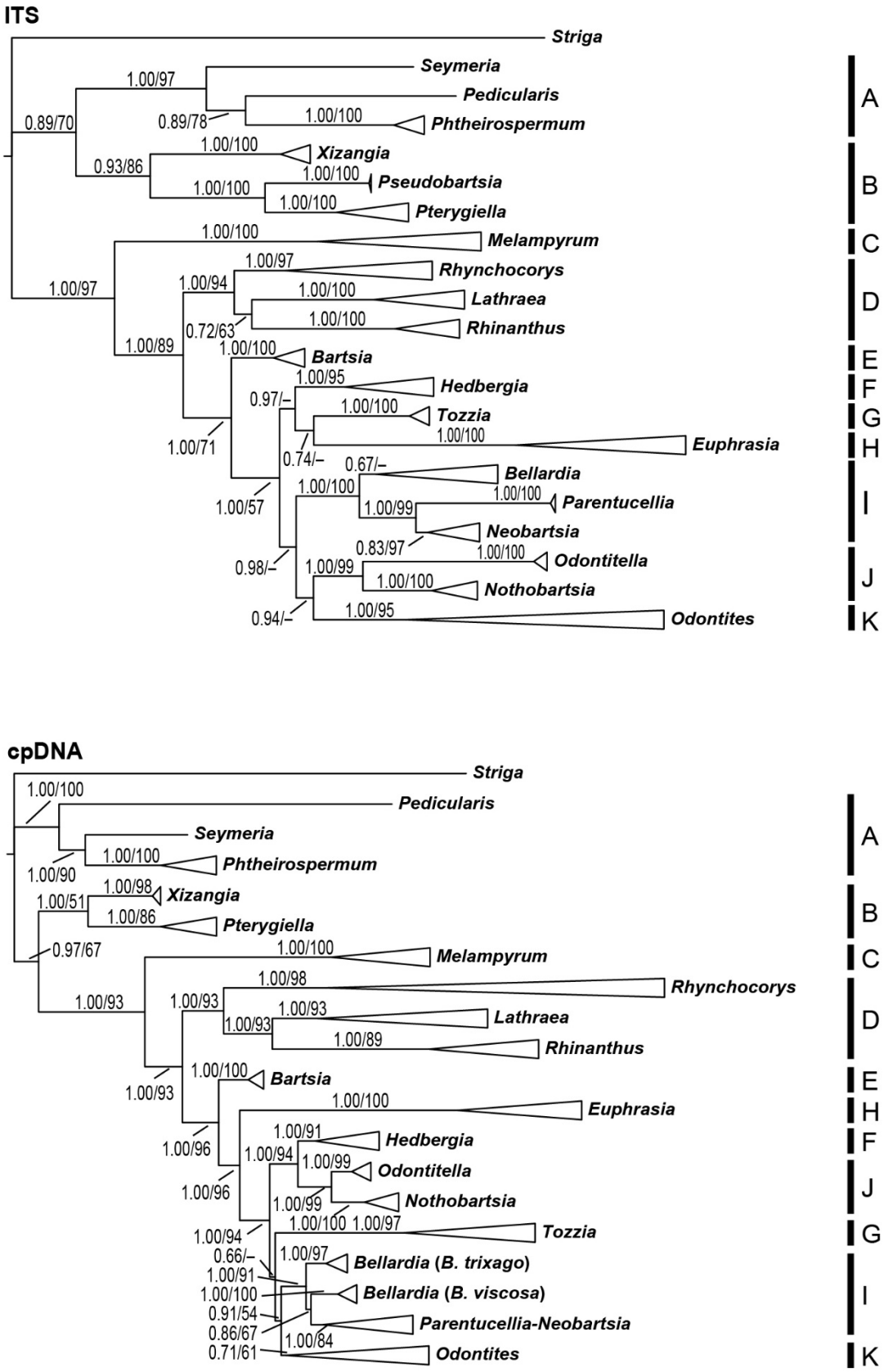

Fig. 3. Majority-rule consensus trees from the Bayesian analysis of tribe Rhinantheae ITS and cpDNA datasets, with branch support (PP/BS). Main clades are indicated with different letters. Clade names: A, tribe Pedicularideae, B, Pterygiella Complex II, C, Melampyrum, D, RRL, E, Bartsia s.str., F, Hedbergia, G, Tozzia, H, Euphrasia, I, Bellardia, J, Nothobartsia-Odontitella, K, Odontites. Clades corresponding to genera were collapsed.

recovered as sister to clades F–K in cpDNA. On the other hand, clade J (ITS PP = 1; BS = 99, cpDNA PP = 1; BS = 99) was sister to *Hedbergia* (clade F) in the cpDNA tree, but sister to *Odontites* s.l.+*Macrosyringion* (clade K) in the ITS analysis (PP = 0.94; BS < 50).

The tribe Rhinantheae s.str. (clades C–K; clade V in McNeal & al., 2013) was strongly supported in both analyses (ITS PP = 1; BS = 97, cpDNA PP = 1; BS = 93). The branching pattern of clade C (*Melampyrum*), clade D ("RRL clade", composed of *Rhinanthus*, *Lathraea* L. and *Rhynchocorys*) and the "core group of Rhinantheae" (clades E–K) was identical to that in Scheunert & al. (2012). *Bartsia alpina* L. (clade E) was the first-branching taxon within the core group of Rhinantheae. Leaving aside incongruent clades (clades H and J), the remaining taxa were grouped into two clades in the ITS tree, the first containing *Hedbergia* and *Tozzia* (clades F and G), and the second grouping together the Bellardia and Odontites clades (clades I and K, respectively). All samples of *Tozzia* formed a clade (clade G; ITS PP = 1; BS = 100; cpDNA PP = 1; BS = 97). In the cpDNA tree, the position of *Tozzia* remained unresolved, as the branch that connected it with clade I-Bellardia plus clade K-Odontites had low support. Clade I-Bellardia was sister to clade K-Odontites in the cpDNA tree (PP = 0.91; BS = 54). Finally, all species of *Odontites* formed a clade (clade K) in both analyses, but with low support in the cpDNA tree (ITS PP = 1; BS = 95, cpDNA PP = 0.71; BS = 61).

Phylogenetic relationships among species of *Odontites* s.l. (clade K). — In the ITS tree, the clade K.2-Macrosyringion (grouping *O. longiflorus* and *O. glutinosus*) appeared nested within *Odontites*, but was recovered as sister to the remaining *Odontites* in cpDNA (Fig. 4, clade K.2; ITS PP = 1; BS = 100; cpDNA PP = 1; BS = 100). Five main lineages were detected in the ITS analysis, and confirmed in the cpDNA tree (Figs. 4, 5). Relationships among these five lineages remained unclear due to low support and short lengths of some internal branches, especially in the ITS analysis. The two species included in the K.1-Pyrenaeus clade (ITS PP = 1; BS = 100; cpDNA PP = 1; BS = 100), *O. cebennensis* H.J.Coste & Soulié and *O. pyrenaeus* (Bubani) Rothm., were monophyletic according to the ITS tree. However, in the cpDNA tree, only the samples corresponding to the former were recovered as a clade, whereas the later formed a grade. The well-supported K.2-Macrosyringion clade was composed of two species, both recovered as monophyletic. In the K.3-Bornmuellerantha clade (ITS PP = 1; BS = 97; cpDNA PP = 1; BS = 100), it was not possible to test the reciprocal monophyly of its two species as only one sequence of *O. alshehbazianus* (Dönmez & Mutlu) A.Fleischm. & Heubl was analysed. The K.4-Viscosus clade contains a higher number of taxa compared to clades K.1 to K.3. The K.4 clade was monophyletic in the ITS tree (PP = 1; BS = 95), but divided into two subclades in the cpDNA tree, with one being sister to the K.1-Pyrenaeus clade. However, given that the branch that grouped K.1 with this

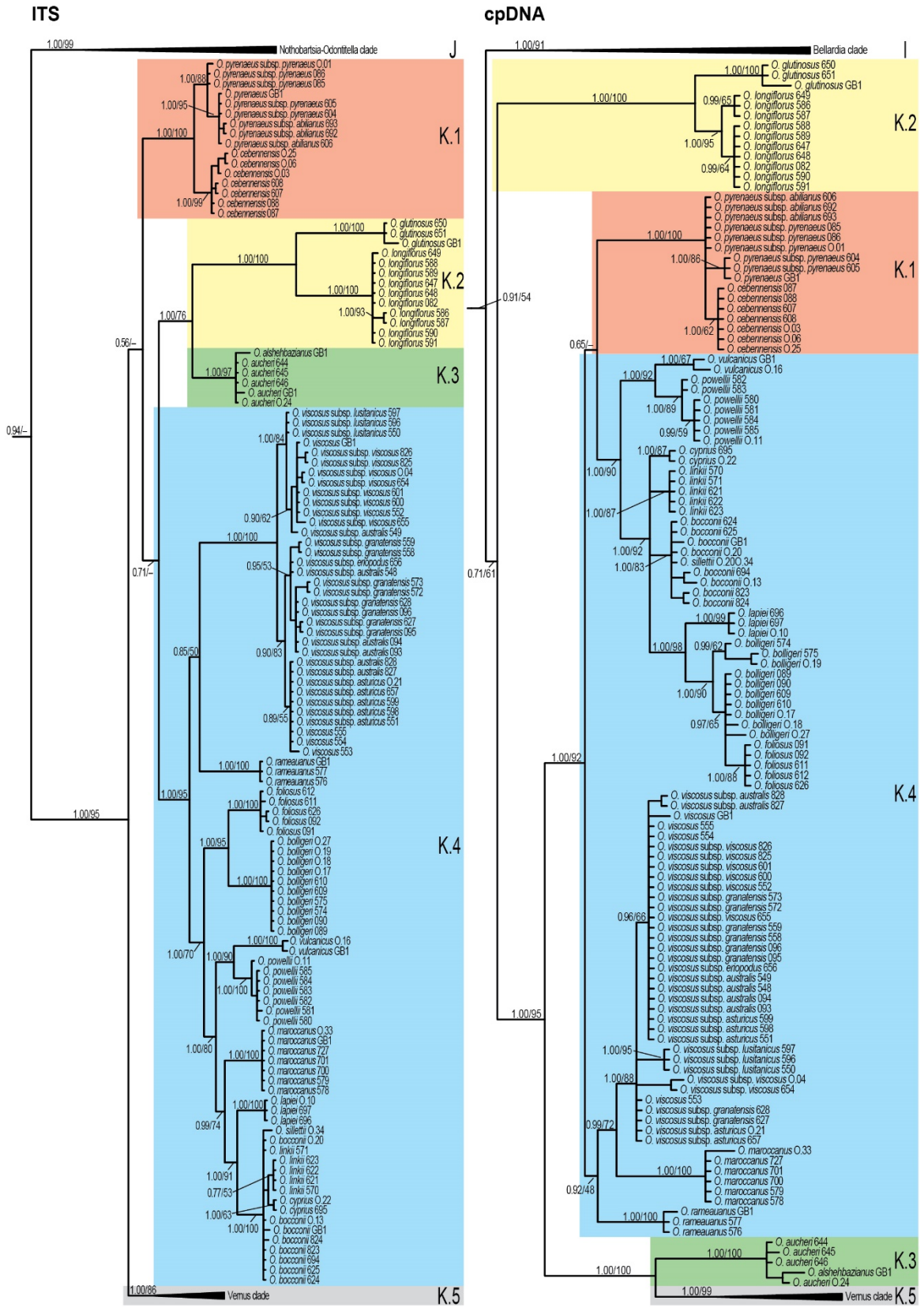

Fig. 4. Majority-rule consensus trees from the Bayesian inference analysis, with branch support (PP/BS), showing the main clades of *Odontites* and sister clades (J-Nothobartsia-Odontitella, I-Bellardia) based on ITS and cpDNA. Main lineages are indicated with different colours and letters. Clade names: K.1-Pyrenaeus, K.2-Macrosyringion, K.3-Bornmuellerantha and K.4-Viscosus. Clade K.5-Vernus was collapsed. Ma., *Macrosyringion*; O., *Odontites*; Synonyms: *O. cyprius* = *O. linkii* subsp. *cyprius*; *O. glutinosus* = *Ma. glutinosum*; *O. longiflorus* = *Ma. longiflorum*.

K.4 subclade had low support (PP = 0.65; BS < 50), the topologies of the two trees showed a soft incongruence and became compatible when a branch collapsing threshold of PP < 0.80 was applied. Most of the species sampled of the K.4-Viscosus clade proved to be monophyletic (with two exceptions; i.e., *O. bolligeri* E.Rico & al. in the cpDNA tree, and the species belonging to the "Bocconii group" sensu Bolliger, 1996). Finally, of the large number of taxa in the K.5-Vernus clade (ITS PP = 1; BS = 86; cpDNA PP = 1; BS = 99), which have been described based on morphology, only four narrowly endemic species formed monophyletic groups: *O. corsicus* (Loisel.) G. Don. (in ITS only), *O. hollianus* (Lowe) Benth., *O. kaliformis* (Pourr. ex Willd.) Pau, and *O. recordonii* Burnat & Barbey.

Several cases of topological incongruence were detected at both the intrageneric and interspecific levels. At the intrageneric level (Fig. 6A), the K.3-Bornmuellerantha clade was sister to the K.2-Macrosyringion clade in the ITS tree (PP = 1; BS = 76), but sister to the K.5-Vernus clade in the cpDNA tree (PP = 1; BS = 100). At the interspecific level, there were two cases of incongruence, both in the K.4-Viscosus clade (Fig. 6B). The first case concerned *O. maroccanus* Bolliger. It was sister to *O. viscosus* (L.) Clairv. in the cpDNA tree (PP = 0.99; BS = 72), but sister to a clade containing *O. lapiei* Batt. and the Bocconii-linkii clade (PP = 0.99; BS = 74) in the ITS tree. The second incongruence concerned a clade that included two species: *O. bolligeri* and *O. foliosus* Pérez Lara. This clade was sister to *O. lapiei* in the cpDNA tree (PP = 1; BS = 98), but part of a clade that included most of the North African and Central to Eastern Mediterranean species (PP = 1; BS = 70) in ITS.

DISCUSSION

Delimitation of tribe Rhinantheae. — Here, the most comprehensive phylogeny of tribe Rhinantheae to date, including two or more samples of all recognized genera, is presented. All major clades revealed in previous ITS phylogenies were confirmed as well as most of the clades recovered in previous cpDNA studies (Scheunert & al., 2012; Dong & al., 2013; McNeal & al., 2013; Uribe-Convers & Tank, 2015; Gaudeul & al., 2016). The monophyly of tribe Rhinantheae (sensu McNeal & al., 2013, i.e., including the East Asian *Pterygiella* complex II) was not supported by our ITS tree, as the *Pterygiella* complex II was recovered as sister to tribe Pedicularideae (Fig. 3A, clades B and A respectively). In the cpDNA analysis, it was sister to Rhinantheae s.str., although both groups were separated by long branches (i.e., large genetic distances). This topological incongruence had previously been detected by McNeal & al. (2013) and Zhou & al. (2014), although they did not explicitly comment on it. McNeal & al. (2013) even proposed a node-based definition of the Rhinantheae that omitted this finding. The monophyly

of the *Pterygiella* complex II has been clearly demonstrated (Dong & al., 2013), and the observed topological incongruence casts its inclusion in tribe Rhinantheae into doubt. Additionally, the members of this complex have at least two morphological characters that differentiate them from those of tribe Rhinantheae s.str. (clade V in McNeal & al., 2013). First, they have a five-toothed instead of a four-toothed calyx as present in the Rhinantheae (Molau, 1988, 1990; Hong & al., 1998; Benedí & al., 2009). Second, all species in the *Pterygiella* complex II have pollen grains of types Ia-1 or IV (with granulate or regularly retipilate exine sculpturing, and a size of <27 μm; Lu & al., 2007), while in tribe Rhinantheae s.str. pollen grains have variable exine surfaces and sizes >27 μm (except in *Tozzia*; İnceoğlu, 1982; Minkin & Eshbaugh, 1989; Bolliger & Wick, 1990; Lu & al., 2007). Further studies are necessary to shed light on the tribal placement of *Pseudobartsia*, *Pterygiella* and *Xizangia*, using an adequate selection of markers and samples.

Our results validate the polyphyly of *Phtheirospermum*, as already suggested by various authors (Dong & al., 2013; McNeal & al., 2013; Zhou & al., 2014). The generitype species, *Ph. japonicum* (= *Ph. chinense* Bunge ex Fisch. & C.A.Mey.), clustered within tribe Pedicularideae (clade A-Pedicularideae), but the other three species of *Phtheirospermum* formed a clade together with *Pterygiella* (clade B-*Pterygiella* complex II; see Fig. S1). As recommended by McNeal & al. (2013), the most conservative option is to include *Ph. muliense* C.Y.Wu & D.D.Tao, *Ph. parishii* Hook.f., and *Ph. tenuisectum* Bureau & Franch. in *Pterygiella*. The following new combinations are therefore required:

Pterygiella muliensis (C.Y.Wu & D.D.Tao) Pinto-Carrasco, E.Rico & M.M.Mart.Ort., **comb. nov.** ≡ *Phtheirospermum muliense* C.Y.Wu & D.D.Tao in Acta Bot. Yunnan. 18: 307, fig. 4. 1996.

Pterygiella parishii (Hook.f.) Pinto-Carrasco, E.Rico & M.M.Mart.Ort., **comb. nov.** ≡ *Phtheirospermum parishii* Hook.f., Fl. Brit. India 4: 304. 1884.

Pterygiella tenuisecta (Bureau & Franch.) Pinto-Carrasco, E.Rico & M.M.Mart.Ort., **comb. nov.** ≡ *Phtheirospermum tenuisectum* Bureau & Franch. in J. Bot. (Morot) 5: 129. 1891.

Relationships within tribe Rhinantheae. — Relationships among genera in tribe Rhinantheae s.str. as presented here are very similar to those previously reported. The position of the most basal clades (clade C-Melampyrum, clade D-RRL and clade E-Bartsia s.str.) seems to be undisputed, as the same topology was recovered with high support in most studies (Těšitel & al., 2010; Scheunert & al., 2012; McNeal & al., 2013, Uribe-Convers & Tank, 2015), with only one exception (Gaudeul & al., 2016), probably due to incorrect outgroup selection. The

branching order of the remaining genera is more questionable. The presence of short branches, poorly supported nodes and incongruence among markers results in discordant evolutionary hypotheses among studies. One of the most controversial points is the position of *Tozzia*. Our study is the first to analyse more than one sample (including *T. alpina* subsp. *carpathica* (Woł.) Pawł. & Jasiewicz = *T. carpathica* Woł.) of *Tozzia*, confirming its monophyly. With respect to this genus, our topologies disagree with three previously published phylogenies (McNeal & al., 2013; Uribe-Convers & Tank 2015; Gaudeul & al., 2016; Fig. 1), but agree with Scheunert & al. (2012). In all cases, the phylogenetic relationships of *Tozzia* are not statistically supported (in both nrDNA and cpDNA trees). Furthermore, the morphology and life form of *Tozzia* is so different from that of the phylogenetically most closely related genera [e.g., unilocular one-seeded and slightly fleshy indehiscent (sometimes dehiscent by late ripening) fruits vs. bilocular many-seeded dry capsules] that it is not possible to make a suggestion concerning its relationships. The evolutionary history of *Tozzia* needs more thorough study.

Concerning clade J, our results agree with previous phylogenies (Scheunert & al., 2012; Gaudeul & al., 2016) placing the Iberian endemic *Odontitella* as sister to, however clearly differentiated from, the Iberian subendemic *Nothobartsia*. Despite the great number of shared character states between *Odontitella* and *Nothobartsia* (e.g., strongly bilabiate corolla with entire galea, papillose pubescence of stamen filaments, mucronate anthers, retipilate pollen exine sculpturing, long-stalked calycinal glandular hairs absent; Bolliger & Molau, 1992), only a few of them differentiate this clade from *Odontites* (clade K), due to the high morphological variability in the latter genus. Diagnostic characters include the stem indument, which in the members of clade J is composed only of eglandular antrorse (sometimes antrorse to patent) hairs, while this specific type of hair is never present in *Odontites* (Benedí, 2009; Rico, 2009). Additionally, corolla colour darkens during anthesis, from yellow to brownish-red, in *Odontitella virgata* (Link) Rothm. and *N. asperrima* (Link) Benedí & Herrero (not observable in *N. spicata* (Ramond) Bolliger & Molau as its corolla is invariably purple-violet), while it has never been found to change in any species of *Odontites*. On the other hand, there are several characters that separate *Odontitella* and *Nothobartsia*. *Odontitella* is an annual, with entire to few-toothed, linear to narrowly lanceolate leaves, bracts resembling the leaves, corolla tube shorter than (or as long as) the calyx, and style clavate beneath the stigma, while *Nothobartsia* is a perennial, with crenate to dentate, broadly ovate or elliptical leaves, bracts not resembling the leaves, corolla tube longer than calyx, and style not clavate beneath the stigma. The inclusion of *Nothobartsia* in *Odontitella* is therefore not supported by morphological data, and phylogenetic data do not provide direct evidence to merge them. Thus, we propose to maintain them as separate

genera.

Delimitation and main lineages of *Odontites* (clade K). — The delimitation of *Odontites* has long been controversial. The topologies presented here generally agree with those of Scheunert & al. (2012), but disagree with the cpDNA tree obtained by Gaudeul & al. (2016). In the latter study, *Macrosyringion* was recovered as sister to a clade composed by *Bellardia* and *Parentucellia*, with high support. However, based on our results, and in contrast to the taxonomy used by these authors, we propose that *M. longiflorum* (Lam.) Rothm. and *M. glutinosum* (M.Bieb.) Rothm. should be transferred back to *Odontites*. Consequently, our clade-based definition of *Odontites* is: the least inclusive crown clade containing *O. pyrenaeus* (Bubani) Rothm., *O. longiflorus* (Lam.) G.Don., *O. aucheri* Boiss., *O. viscosus* (L.) Clairv., and *O. vernus* (Bellardi) Dumort. (= *Euphrasia odontites* L.; type of the genus name).

The reinclusion of *Macrosyringion* in *Odontites* does not involve any nomenclatural changes, as validly published combinations already exist for these taxa: *O. longiflorus* (Lam.) G.Don and *O. glutinosus* (M.Bieb.) Benth. Furthermore, as samples of *O. longiflorus* subsp. *lateritius* (Charpin & Fern. Casas) Sánchez-Gómez and *O. longiflorus* var. *roseus* A.Segura did not genetically differ from other samples of *O. longiflorus*, and morphological differences among them are superficial, we do not consider them separate taxonomic entities.

The five main lineages of *Odontites* can be distinguished morphologically using characters related mainly to the flower and inflorescence (Fig. 2). Table 2 summarizes the states of several morphological traits present in the species of the respective lineages. The morphological characterization of these five lineages is based on the comparison of the extensive descriptions found in the monograph by Bolliger (1996), *Flora iberica* (Rico, 2009), as well as in the papers where new taxa were described (Dönmez & Mutlu, 2010; Brullo & al., 2015). The morphologically most distinct lineages are clade K.2-Macrosyringion and clade K.3-Bornmuellerantha, which have at least five and three morphological synapomorphies, respectively; by contrast, the taxa belonging to clade K.4-Viscosus have only one (and a second character state is almost synapomorphic). The K.1-Pyrenaeus and K.5-Vernus lineages share at least nine character states that, despite not being unique, are useful in combination to distinguish these from the remaining lineages. However, in our opinion, the only characters which unambiguously differentiate those two lineages from each other are cell number, orientation of cellular divisions, and outline of the head of the calycinal glandular hairs. In the Lanceolata and Kaliformis-types (present in only some species of the K.5-Vernus clade) of long-stalked glands (Bolliger, 1985), the general arrangement of the glandular head (sphaerical, with two to 16 cells,

derived mainly from longitudinal divisions) is almost identical to that of the typical short-stalked glandular hairs differing only in length. In our view, the Lanceolata and Kaliformis-type glands should be reclassified to be included into the variability of the short-stalked glands. Therefore, all members of the K.5-Vernus clade lack long-stalked calycinal glands, while those belonging to the K.1-Pyrenaeus clade show Pyrenaea-type glands (ellipsoid to sphaerical and composed of a much higher number of cells [30–200], which derive from both transversal and longitudinal divisions).

A widely used character, at least in dichotomous keys (e.g., Webb & Camarasa, 1972; Bolliger, 1996), is corolla colour. However, its usefulness is dubious. Fixed colours are found only in the K.3-Bornmuellerantha and K.1-Pyrenaeus lineages, which consistently have yellow corollas. Nevertheless, there exist species in all other lineages sharing this character state (Fig. 2). Within lineages, corolla colour is useful in K.5-Vernus as stated below. At the species level, corolla colour is almost constant in most cases. In *O. longiflorus*, *O. jaubertianus*, and *O. viscosus*,

Table 2. Morphological characters for differentiation of lineages or groups of species.

	Lineage				
	K.2-Macrosyringion	K.3-Bornmuellerantha	K.4-Viscosus	K.1-Pyrenaeus	K.5-Vernus
Glandular hairs at base of stem	**present (0.2–0.3 mm)**	absent	absent (present – 0.5 to 3.0 mm – in *O. viscosus**)	absent	
Long-stalked glands on calyx	**present (longiflora type)**	present (pyrenaea type)	present (rameauana, pyrenaea and viscosa types) or absent	present (pyrenaea type)	absent
Corolla shape	strongly bilabiate	**subrotate**	strongly bilabiate	strongly bilabiate	
Corolla hairs	**glandular plus eglandular**	eglandular (restricted to lips)	**absent or almost absent (tube with eglandular hairs in *O. rameauanus*)**	eglandular	
Corolla tube	**long (2–5 times longer than calyx)**	short (slightly shorter or longer than calyx)	short (slightly shorter or longer than calyx)	short (slightly shorter or longer than calyx)	
Corolla upper lip	straight	-	**folded downwards**	straight	straight or folded upwards*
Theca base	mucronate	**obtuse**	mucronate	mucronate	
Pollen exine	retipilate	**retirugulate**	microreticulate or retipilate	microreticulate	
Stigma shape	**bilobate**	entire	entire	entire	
Habit	annual	annual	annual or perennial*	annual	
Growth form	erect	erect	erect or prostrate	erect	erect or prostrate
Inflorescence type	acropetal	acropetal	acropetal or basipetal*	acropetal	
Toothed bracts	no	no	no	yes or no	
Corolla colour	yellow or very rarely pink	yellow	yellow, pink, dark red* or bicoloured*	yellow	yellow or pink
Stamen filament pubescence	papillose	glabrous	papillose or glabrous	papillose	papillose or pilose* (glabrous in *O. corsicus*)
Seed number	17–40	10–12	4*–20	8–14	12–40
Chromosome number	22–24–26	24	20–22–24–26	24	18*–20–22–24–26–40*

Synapomorphies for each lineage are in bold.
* Character state present in only one lineage, but not shared by all species.

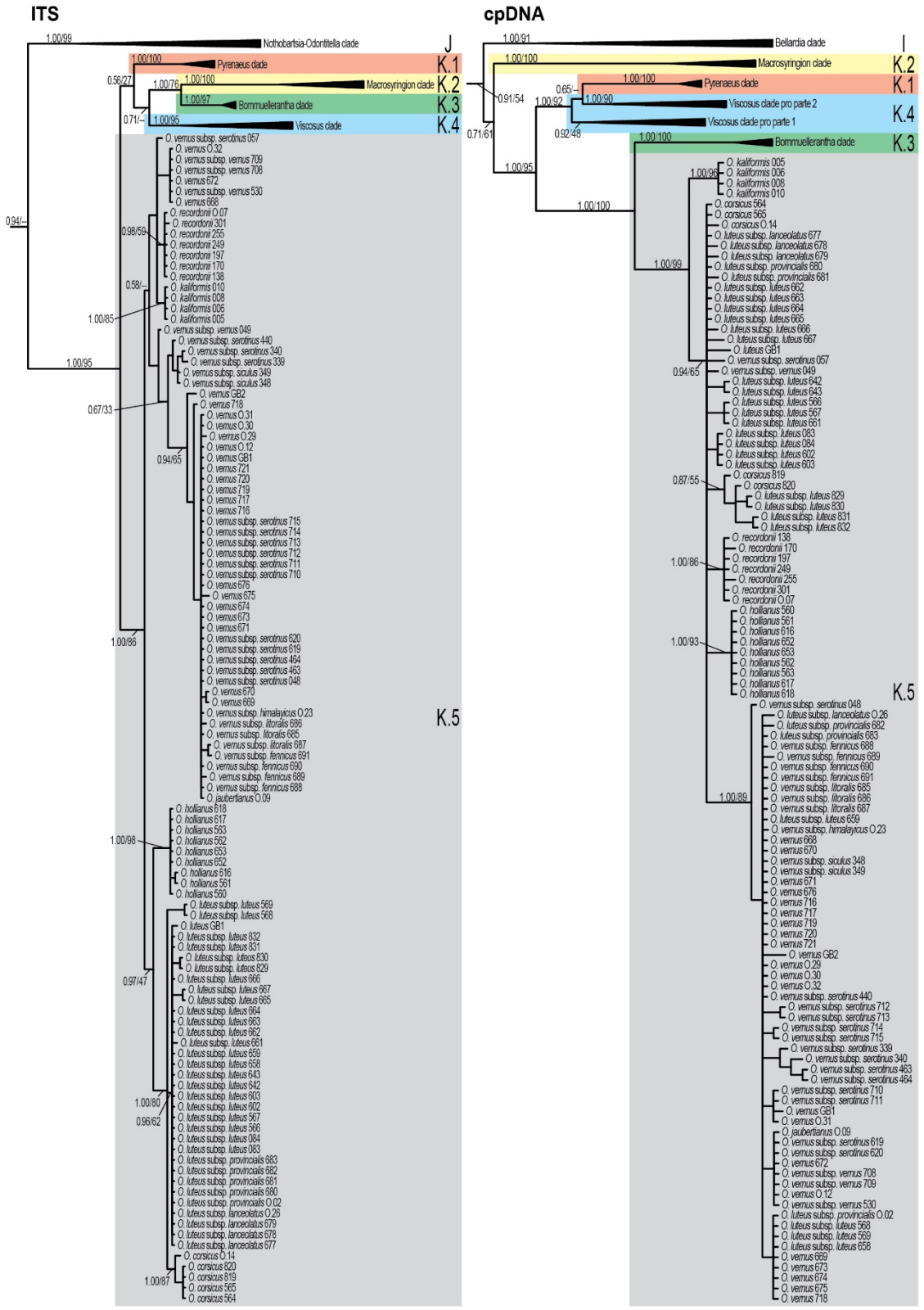

Fig. 5. Majority rule consensus trees from the Bayesian inference analysis, with branch support (PP/BS), showing the K.5- Vernus clade based on A) ITS and B) cpDNA. Other clades belonging to *Odontites* (K.1- Pyrenaeus, K.2- Macrosyringion, K.3- Bornmuellerantha and K.4- Viscosus) and sister clades (J- Nothobartsia-Odontitella, I- Bellardia) were collapsed. O. = *Odontites*. Synonyms: *O. luteus* subsp. *lanceolatus* = *O. lanceolatus* subsp. *lanceolatus*; *O. luteus* subsp. *provincialis* = *O. lanceolatus* subsp. *provincialis*; *O. vernus* subsp. *fennicus* = *O. litoralis* subsp. *fennicus*; *O. vernus* subsp. *himalayicus* = *O. vulgaris* subsp. *himalayicus*; *O. vernus* subsp. *litoralis* = *O. litoralis* subsp. *litoralis*; *O. vernus* subsp. *serotinus* = *O. vulgaris* subsp. *vulgaris*; *O. vernus* subsp. *siculus* = *O. vulgaris* subsp. *siculus*.

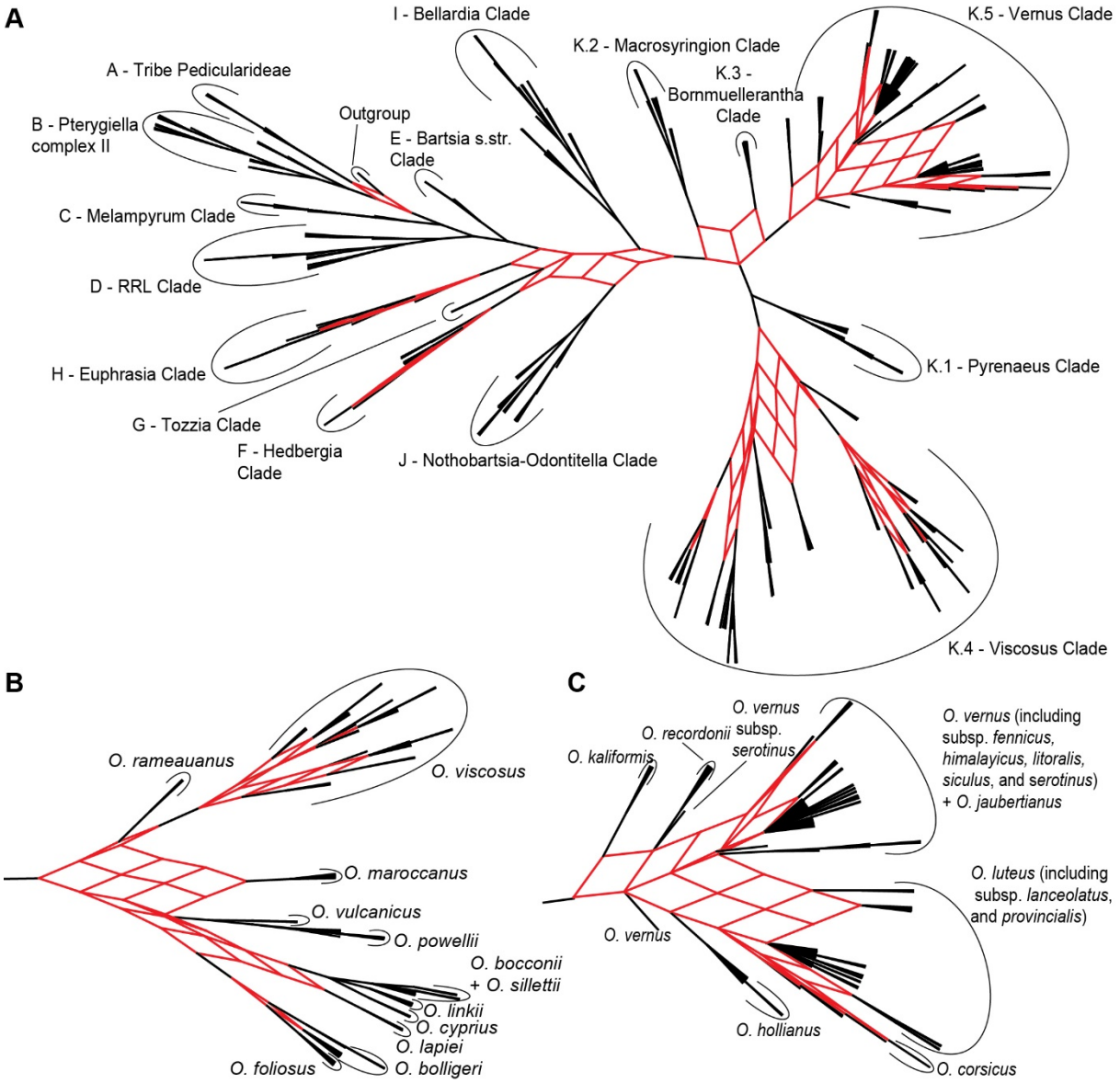

Fig. 6. SuperNetwork obtained from the Bayesian inference trees (branches collapsed at PP < 0.8) illustrating the incongruence between ITS and cpDNA topologies. Branch lengths were not taken into account. Clades are named as in Figs. 2–4. **A,** tribe Rhinantheae; **B,** K.4-Viscosus clade; **C,** K.5-Vernus clade. Ma., *Macrosyringion*; O. = *Odontites*. Synonyms: *O. cyprius* = *O. linkii* subsp. *cyprius*; *O. luteus* subsp. *Lanceolatus* = *O. lanceolatus* subsp. *lanceolatus*; *O. luteus* subsp. *provincialis* = *O. lanceolatus* subsp. *provincialis*; *O. vernus* subsp. *fennicus* = *O. litoralis* subsp. *fennicus*; *O. vernus* subsp. *himalayicus* = *O. vulgaris* subsp. *himalayicus*; *O. vernus* subsp. *litoralis* = *O. litoralis* subsp. *litoralis*; *O. vernus* subsp. *serotinus* = *O. vulgaris* subsp. *vulgaris*; *O. vernus* subsp. *siculus* = *O. vulgaris* subsp. *siculus.*

however, this character is polymorphic (yellow and pink in the first two species, and a gradient from yellow to dark red in the latter; Fig. 2). Taking into account these facts, corolla colour can be useful as the main character to differentiate groups of species only in particular cases (e.g., K.5-Vernus clade).

Although it was not possible to include samples of several narrowly endemic species from North Africa, Sicily, and the Near East in our study, we can place them preliminarily into our lineages, using the cpDNA data from Gaudeul & al. (2016). *Odontites hispidulus* (Boiss.) Bolliger, endemic to Lebanon and Syria, is morphologically very similar to *O. luteus* (L.)

Clairv. (Bolliger, 1996). The species was recovered within clade B2 by Gaudeul & al. (2016), which is part of our K.5-Vernus lineage. Based on this evidence, we tentatively consider *O. hispidulus* to be a member of the K.5-Vernus clade. All but one North African species (i.e., *O. discolor* Pomel, *O. purpureus* (Desf.) G.Don, *O. triboutii* Gren. & Paill. and *O. violaceus* Pomel), and the Sicilian *O. rigidifolius* (Biv.) Benth., which were not sampled here, in Gaudeul & al. (2016) were part of a highly supported clade with *O. vulcanicus* Bolliger, *O. powellii* Maire, *O. linkii* Heldr. & Sart. ex Boiss., *O. bocconii* (Guss.) Walp. and *O. lapiei*. All of these taxa are found within our K.4-Viscosus lineage; thus, the unsampled species are likely to be part of K.4-Viscosus as well. The only North African species for which phylogenetic affinities remain unknown is the Tunisian endemic *O. citrinus* Bolliger. The morphologically most similar and geographically nearest species is *O. triboutii* (endemic from NE Algeria and Tunisia), and together they form the Triboutii-group (Bolliger, 1996). Therefore, we tentatively include *O. citrinus* in our K.4-Viscosus lineage. A list of taxa included in each lineage is provided in Table 3.

Species monophyly, hybridization, and ILS: Assessing taxonomic boundaries within *Odontites*. — By including at least two samples per species, we were able to test the monophyly of the vast majority of species studied. Within *Odontites*, only three species (*O. alshehbazianus*, *O. jaubertianus* and *O. sillettii* Brullo & al.) were represented by a single sequence, and the monophyly of them thus remains undemonstrated. Of the remaining 23 species (78%), 18 were monophyletic and were recovered as well-supported clades in at least one dataset (most of them in both datasets). All of them are recognized here as valid species (Table 3). The monophyly of *O. linkii* (as circumscribed by Bolliger, 1996, i.e., including *O. cyprius* Boiss. and *O. creticus* Boiss.) was not supported. In the cpDNA analysis, these sequences formed two clades that corresponded to the samples collected in Cyprus and the Peloponnese, respectively. Additionally, the samples from Cyprus were also recovered as monophyletic in the ITS tree, which encourages us to recognize the taxon as a separate species under the resurrected name *O. cyprius*. The taxonomic status of *O. creticus* remains unknown.

Regarding *Odontites hollianus*, the samples from Madeira Island (Madeira Archipelago) were molecularly indistinguishable from those collected in La Palma Island (Canary Islands). Morphologically, the Canary Islands plants are very similar to those from Madeira, but there is a slight difference in stamen morphology that has been considered taxonomically useful (i.e., Rothmaler, 1943; Bolliger, 1996). The papillae on the stamen filaments are ca. 45 μm long in the Canary Island samples, instead of ca. 30 μm in the Madeiran material. Despite their clear isolation by distance (ca. 500 km), the incipient morphological differentiation is not sufficient to

Table 3. Taxa assigned to particular phylogenetic lineages.

Lineage	Species and subspecies of *Odontites* Ludw.
K.1-Pyrenaeus	*O. cebennensis* H.J.Coste & Soulié
	O. pyrenaeus (Bubani) Rothm. subsp. *pyrenaeus*
	O. pyrenaeus subsp. *abilianus* P.Monts.
K.2-Macrosyringion	*O. glutinosus* (M.Bieb.) Benth.
	O. longiflorus (Lam.) G.Don
K.3-Bornmuellerantha	*O. alshehbazianus* (Dönmez & Mutlu) A.Fleischm. & Heubl
	O. aucheri Boiss.
K.4-Viscosus	*O. bocconii* (Guss.) Walp.
	O. bolligeri E.Rico & al.
	* *O. citrinus* Bolliger
	O. cyprius Boiss.
	* *O. discolor* subsp. *ciliatus* (Pomel) Bolliger
	* *O. discolor* Pomel subsp. *discolor*
	O. foliosus Peréz Lara
	O. lapiei Batt.
	O. linkii Heldr. & Sart. ex Boiss.
	O. maroccanus Bolliger
	O. powellii Maire
	* *O. purpureus* (Desf.) G.Don
	O. rameauanus Emb.
	* *O. rigidifolius* (Biv.) Benth.
	O. sillettii Brullo & al.
	* *O. triboutii* Gren. & Paill.
	* *O. violaceus* Pomel
	O. viscosus subsp. *asturicus* M.Laínz
	O. viscosus subsp. *australis* (Boiss.) Jahand. & Maire
	O. viscosus subsp. *eriopodus* Litard. & Maire
	O. viscosus subsp. *granatensis* (Boiss.) Bolliger
	O. viscosus subsp. *lusitanicus* Bolliger
	O. viscosus (L.) Clairv. subsp. *viscosus*
	O. vulcanicus Bolliger
K.5-Vernus	*O. corsicus* (Loisel.) G.Don.
	* *O. hispidulus* (Boiss.) Bolliger
	O. hollianus (Lowe) Benth.
	O. jaubertianus (Boreau) D.Dietr.
	O. kaliformis (Pourr. ex Willd.) Pau
	O. luteus subsp. *lanceolatus* (Gaudin) P.Fourn.
	O. luteus (L.) Clairv. subsp. *luteus*
	O. luteus subsp. *provincialis* (Bolliger) J.-M.Tison
	O. recordonii Burnat & Barbey
	O. vernus subsp. *fennicus* (Markl.) Pinto-Carrasco & al.
	O. vernus subsp. *himalayicus* (Pennell) Pinto-Carrasco & al.
	O. vernus subsp. *litoralis* (Fr.) Nyman
	* *O. vernus* subsp. *mesatlanticus* (Emb. & Maire) Pinto-Carrasco & al.
	O. vernus subsp. *serotinus* Corb.
	O. vernus subsp. *siculus* (Guss.) Sell
	O. vernus (Bellardi) Dumort. subsp. *vernus*

* Not included in our molecular analysis. Inclusion in its lineage based on morphological similarity and/or results by Gaudeul & al. (2016).

segregate the Canary Island populations in a new taxon (subspecies or species). Further studies using adequate molecular tools (e.g., microsatellites; Pinto-Carrasco & al., 2016) are necessary to investigate its genetic isolation and re-evaluate our conservative taxonomic treatment.

By comparing the trees obtained from the ITS and cpDNA datasets, we detected incongruences at three levels (lineages, species, and individuals). The backbones of the ITS and cpDNA trees show short branches and low support (Figs. 3 and 4). This situation is consistent with a scenario where several lineages evolved in a short time (Wortley & al., 2005). The soft incongruence affecting the K.1-Pyrenaeus and K.4-Viscosus clades could be the result of such a scenario, since each genetic marker could reveal a slightly different evolutionary history. Concerning the phylogenetic relationships among the clades K.3-Bornmuellerantha, K.5-Vernus, and K.2-Macrosyringion observed in the trees, two alternative hypotheses involving ancient homoploid hybridization could be postulated: (A) ancestors of the K.5-Vernus and K.2-Macrosyringion clades hybridized to generate the K.3-Bornmuellerantha clade; or (B) the K.2-Macrosyringion clade was the result of an interspecific cross between the ancestor of the K.3-

Bornmuellerantha clade and a currently extinct taxon that would be the ancestor of the extant *Odontites*. Supporting the first option, species belonging to clades K.5 and K.2 are present where the taxa of K.3 grow today (Near East, from Turkey to the Caspian Sea; Bolliger, 1993, 1996). On the other hand, the low support for the sister relationship of clade K.2 and the remaining clades (K.1, K.3–5) in the cpDNA tree are in agreement with the second hypothesis as this could be the result of not sampling its extinct maternal parent. Morphologically, K.3-Bornmuellerantha and K.2-Macrosyringion are the two most divergent clades (three and five synapomorphies, respectively; Table 2), which blurs their phylogenetic relationships. The choice of either hypothesis would be merely speculative; further studies are needed to clarify this issue. In case reticulate evolutionary processes should have led to the formation of *O. maroccanus* and *O. bolligeri–O foliosus* (and thus would have caused the observed incongruence between nuclear and plastid trees), they might have occurred more recently than those of the Bornmuellerantha/ Macrosyringion clades, as the presumed parents should have been members of the K.4-Viscosus clade. The node ages recently published by Gaudeul & al. (2016) corroborate our findings. According to these authors, the K.3-Bornmuellerantha clade diverged from the K.5-Vernus clade around 10.3 million years ago (mya), while *O. maroccanus* diverged from *O. viscosus*, and *O. bolligeri–O. foliosus* (included under the nomen nudum *O. squarrosus*; Rico & al., 2008) from *O. lapiei* around 4.8 and 2.9 mya, respectively.

Regarding the highly polymorphic *O. viscosus*, not all morphological subspecies constituted molecular clades (Fig. 4). However, the samples were to some extent structured by geography in the ITS tree. This situation might be due to a scenario of recent and/or current gene flow between subspecies in contact zones; this was also postulated using morphological data (gradation in some characters in contact zones; Bolliger, 1996; Rico, 2009). *Odontites viscosus* subsp. *asturicus* M.Laínz and *O. viscosus* subsp. *granatensis* (Boiss.) Bolliger are morphologically and ecologically similar (small few-branched orophytes), but phylogenetically indistinguishable from *O. viscosus* subsp. *australis* (Boiss.) Jahand. & Maire and *O. viscosus* subsp. *eriopodus* Litard. & Maire. Finally, the samples of *O. viscosus* subsp. *lusitanicus* Bolliger group together in both analyses. Despite Rico (2009) considering this subspecies to be part of the huge variability of *O. viscosus* subsp. *australis*, we temporarily reinstate *O. viscosus* subsp. *lusitanicus* until further studies shed light on the validity and the distribution range of all subspecies.

The molecular delimitation of the K.5-Vernus clade as a whole was unambiguous (Fig. 5), but species delimitation within this group was more problematic, likely due to extensive phylogenetic incongruence (Fig. 6C). Only four species within the complex were recovered as monophyletic and are therefore accepted here (*O. corsicus*, *O. hollianus*, *O. kaliformis*, and *O.*

recordonii). The remaining three species (i.e., *Odontites vernus* s.l., *O. luteus* s.l., *O. jaubertianus*) were completely intermixed in the cpDNA tree. They form two morphological groups of taxa with different corolla colour and floral morphology (*O. vernus* and *O. luteus* groups; Bolliger, 1996), with *O. jaubertianus* being morphologically intermediate between them due to its allopolyploid origin (Bolliger & al., 1990). In the ITS analyses, relationships among taxa were somewhat clearer, and the K.5-Vernus clade was divided into two subclades associated with corolla colour (yellow in *O. corsicus*, *O. hollianus*, and *O. luteus* s.l., and pink in *O. kaliformis*, *O. recordonii*, *O. jaubertianus* var. *jaubertianus*, and *O. vernus* s.l.). However, two species recognized by Bolliger (1996), *O. litoralis* (Fr.) Fr. (with two subspecies), and *O. vulgaris* Moench. (with four subspecies) remain indistinguishable from *O. vernus*, and the same applies to *O. lanceolatus* (Gaudin) Reichenb. (with two subspecies), which cannot be distinguished from *O. luteus*. That situation could be the result of ILS, and / or recent or current hybridization events. Despite the fact that the already stabilized *O. jaubertianus* is derived from hybridization between *O. vernus* and *O. luteus* (Bolliger & al., 1990), the two latter species are almost completely isolated reproductively. They grow sympatrically in vast areas, but individuals of presumed hybrid origin (intermediate morphology) have very scarcely been reported. No crossing experiments among taxa of the *O. luteus* complex (i.e., subsp. *luteus*, subsp. *lanceolatus* (Gaudin) P. Fourn. and subsp. *provincialis* (Bolliger) J.-M. Tison) have been made. Nevertheless, there is a morphological gradient between the three subspecies (local adaptation to environmental conditions; Tison & al., 2010) and interbreeding could be extensive as they grow in partial sympatry and they have the same chromosome number ($2n = 20$, Bolliger, 1996). Taking into account the high genetic and morphological similarity between the taxa formerly called *O. lanceolatus* subsp. *lanceolatus* and *O. lanceolatus* subsp. *provincialis* Bolliger, we consider that the most conservative taxonomic solution is to include them in *O. luteus*.

Regarding the *O. vernus* complex, the situation is more intricate than in the case of the *O. luteus* complex, as seven taxa (species and subspecies; Bolliger, 1996), two ploidy levels (di- and tetraploid) and two basic chromosome numbers ($x = 9$ and $x = 10$; Delgado & al., 2015) are involved. The only study of intraspecific hybridization in this group (Snogerup, 1983) was performed using diploids (*O. vernus* subsp. *fennicus* (Markl.) Pinto-Carrasco & al., subsp. *litoralis* (Fr.) Nyman and subsp. *serotinus* Corb.; unknown chromosome base number but probably $x = 9$) and tetraploids (*O. vernus* subsp. *vernus*; $x = 10$). Diploids are reproductively compatible (within and between subspecies), but almost complete incompatibility occurs between different ploidy levels. Morphologically, the extremes of the variability range are clearly distinguishable, but the wide variation in both vegetative and reproductive characters hampers the attribution to

subspecies of a great number of individuals (Bolliger, 1996; Rico, 2009). Considering the changes in morphology due to different host plants (Snogerup, 1982), the presence of ecotypes linked to cytotypes (Koutecký & al., 2012), and the probability of recurrent autopolyploidization (Pinto-Carrasco & al., in prep.), we recommend cautious taxonomic treatment, i.e., to include *O. litoralis* and *O. vulgaris* (sensu Bolliger, 1996) in *O. vernus*. In this way, paraphyly could be avoided to some extent as most of the *O. vernus* s.l. samples form one group in the ITS tree. Further studies, using more variable markers, are needed to shed light on the validity of all subspecies that are now included in this complex.

For *O. litoralis* and *O. vulgaris* to be included in the variability of *O. vernus*, the following new combinations are required:

Odontites vernus subsp. ***fennicus*** (Markl.) Pinto-Carrasco, E.Rico & M.M.Mart.Ort., **comb. nov.** ≡ *Odontites litoralis* subsp. *fennicus* Markl. in Acta Soc. Fauna Fl. Fenn. 72(16): 5. 1955 ("*fennica*").

Odontites vernus subsp. ***himalayicus*** (Pennell) Pinto-Carrasco, E.Rico & M.M.Mart.Ort., **comb. nov.** ≡ *Odontites vulgaris* subsp. *himalayicus* (Pennell) Bolliger in Willdenowia 26: 113. 1996 ≡ *Odontites himalayicus* Pennell, Scroph. W. Himal. (Monogr. Acad. Nat. Sci. Philadelphia 5): 98. 1943.

Odontites vernus subsp. ***mesatlanticus*** (Emb. & Maire) Pinto-Carrasco, E.Rico & M.M.Mart.Ort., **comb. nov.** ≡ *Odontites vulgaris* subsp. *mesatlanticus* (Emb. & Maire) Bolliger in Willdenowia 26: 111. 1996 ≡ *Odontites mesatlanticus* Emb. & Maire in Bull. Soc. Hist. Nat. Afrique N. 22: 58.1931.

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APPENDIX 1

Details of sequences (1) newly generated in this study and (2) obtained from GenBank. For newly generated data, we give information about taxon, ID, DNA source, locality, longitude, latitude, collectors, collection number (and/or exsiccatae number), herbarium voucher, and NCBI accession numbers. For sequences obtained from GenBank, we give taxon, ID, NCBI accession numbers and references where they were originally published (see end of Appendix). In both cases accession numbers are shown in the following order: ITS, *trnK* and *rps16*. Unavailable sequences are indicated by a dash (–). The taxon names previous to the taxonomic changes proposed in this paper are shown in brackets. DNA source was silica gel-dried material (s) and herbarium specimens (h). * Coordinates assignment based on locality description; ** silica gel-dried material and voucher specimens were collected in the same location but on different dates.

(1) Sequences produced for this study

Bellardia trixago (L.) All., 629, s, Spain, Cáceres, Poblado del embalse de Gabriel y Galán, –6.12514, 40.22047, *M. Martínez Ortega & X. Giráldez Fernández, MO 6020* (SALA 142078), KX958618, KX959115, KX958866; ***Bellardia trixago***, 630, s, Spain, Cáceres, Poblado del embalse de Gabriel y Galán, –6.12514, 40.22047, *M. Martínez Ortega & X. Giráldez Fernández, MO 6020* (SALA 142078), KX958619, KX959116, KX958867; ***Bellardia trixago***, 698, s, Spain, Burgos, Castrillo de la Vega, –3.78344, 41.65452, *D. Pinto Carrasco, DP 918* (SALA 142076), KX958685, KX959182, KX958933; ***Bellardia trixago***, 699, s, Spain, Burgos, Castrillo de la Vega, –3.78344, 41.65452, *D. Pinto Carrasco, DP 918* (SALA 142076), KX958686, KX959183, KX958934; ***Bellardia viscosa*** (L.) Fisch. & C.A.Mey., 633, s, Spain, Cáceres, between Guijo de Granadilla and Mohedas de Granadilla, –6.18073, 40.22402, *M. Martínez Ortega & X. Giráldez Fernández, MO 6021* (SALA 142079), KX958622, KX959119, KX958870; ***Bellardia viscosa***, 634, s, Spain, Cáceres, between Guijo de Granadilla and Mohedas de Granadilla, –6.18073, 40.22402, *M. Martínez Ortega & X. Giráldez Fernández, MO 6021* (SALA 142079), KX958623, KX959120, KX958871; ***Euphrasia hirtella*** Jord. ex Reut., 702, s, Spain, Ávila, San Martín de la Vega del Alberche, –5.150, 40.431, *E. Rico & V. Lucía, ER 8041* (SALA 142118), KX958689, KX959186, KX958937; ***Euphrasia hirtella***, 703, s, Spain, Ávila, San Martín de la Vega del Alberche, –5.150, 40.431, *E. Rico & V. Lucía, ER 8041* (SALA 142118), KX958690, KX959187, KX958938; ***Nothobartsia asperrima*** (Link) Benedí & Herrero, 615, s, Portugal, Setúbal, Sesimbra, Cabo Espichel, –9.2108, 38.4142, *M. Santos Vicente & al., MS 960* (SALA 123311), KX958604, KX959101, KX958852; ***Nothobartsia asperrima***, 637, s, Portugal, Santarem, Tomar, Algarvias, –8.431, 39.594, *E. Rico, ER 7909* (SALA 123313), KX958626, KX959123, KX958874; ***Nothobartsia asperrima***, 638, s, Portugal, Santarem, Tomar, Algarvias, –8.431, 39.594, *E. Rico, ER 7909* (SALA 123313), KX958627, KX959124, KX958875; ***Nothobartsia asperrima***, 639, s, Portugal, Setúbal, Sesimbra, Cabo Espichel, –9.2108, 38.4142, *M. Santos Vicente & al., MS 960* (SALA 123311), KX958628, KX959125, KX958876; ***Nothobartsia asperrima***, 640, s, Portugal, Setúbal, Vendas de Azeitão, –8.9843, 38.5284, *M. Santos Vicente & al., MS 958* (SALA 123310), KX958629, KX959126, KX958877; ***Nothobartsia asperrima***, 641, s, Portugal, Setúbal, Vendas de Azeitão, –8.9843, 38.5284, *M. Santos Vicente & al., MS 958* (SALA 123310), KX958630, KX959127, KX958878; ***Nothobartsia asperrima***, 821, s, Morocco, Tanger-Tétouan, between Sidi Jel and Beni Bouker, –5.12731, 35.18902, *D. Pinto Carrasco & al., DP 1062* (SALA 156176), KX958708, KX959205, KX958956; ***Nothobartsia asperrima***, 822, s, Morocco, Tanger-Tétouan, between Sidi Jel and Beni Bouker, –5.12731, 35.18902, *D. Pinto Carrasco & al., DP 1062* (SALA 156176), KX958709, KX959206, KX958957; ***Nothobartsia spicata*** (Ramond) Bolliger & Molau, 613, s, Spain, Santander, Peñarrubia, La Hermida, –4.64, 43.26, *E. Rico, ER 7921* (SALA 125801), KX958602, KX959099, KX958850; ***Nothobartsia spicata***, 614, s, Spain, Santander, Peñarrubia, La Hermida, –4.64, 43.26, *E. Rico, ER 7921* (SALA 125801), KX958603, KX959100, KX958851; ***Nothobartsia spicata***, 635, s, Spain, Oviedo, Ribadesella, –5.03, 43.43, *E. Rico, ER 7920* (SALA 125802), KX958624, KX959121, KX958872; ***Nothobartsia spicata***, 636, s, Spain, Oviedo, Ribadesella, –5.03, 43.43, *E. Rico, ER 7920* (SALA 125802), KX958625, KX959122, KX958873; ***Odontitella virgata*** (Link) Rothm., 81, s, Spain, Burgos, Castrillo de la Vega, –3.75889, 41.64505, *D. Pinto Carrasco, DP 14* (SALA 135636), KX958509, KX959006, KX958757; ***Odontitella virgata***, 592, s, Spain, Cádiz, Los Barrios, –5.59, 36.22, *E. Rico, ER 7959* (SALA 136278), KX958581, KX959078, KX958829; ***Odontitella virgata***, 593, s, Spain, Cádiz, Los Barrios, –5.59, 36.22, *E. Rico, ER 7959* (SALA 136278), KX958582, KX959079, KX958830; ***Odontitella virgata***, 594, s, Spain, A Coruña, Santiso, Barazón, –8.00755, 42.85990, *L. Delgado Sánchez & al., LD 1069* (SALA 136280), KX958583, KX959080, KX958831; ***Odontitella virgata***, 595, s, Spain, A Coruña, Santiso, Barazón, –8.00755, 42.85990, *L. Delgado Sánchez & al., LD 1069* (SALA 136280), KX958584, KX959081, KX958832; ***Odontites aucheri*** Boiss., 644, h, Turkey, Erzincan, Sakaltutan Geçidi, 39.12, 39.87, *J. Aldasoro & al., A-2691* (SALA 120807), KX958633, KX959130, KX958881; ***Odontites aucheri***, 645, h, Turkey, Sivas, Dogançal, 38.03, 39.88, *J. Aldasoro & al., A-2783* (SALA 121447), KX958634, KX959131, KX958882; ***Odontites aucheri***, 646, h, Armenia, Ararat, Lusashogh, 44.9653, 39.8597, *M. Oganesian & al., 03-1575* (MA 742689), KX958635, KX959132, KX958883; ***Odontites aucheri***, O.24, h, Armenia, Ararat, Lusashogh, 44.9653, 39.8597, *M. Oganesian & al., 03-1575* (MSB 123864), KX958740, KX959237, KX958988; ***Odontites bocconii*** (Guss.) Walp., 624, s, Italy, Sicily, San Martino delle Scale, 13.2581, 38.0861, *G. Domina, s.n.* (PAL 90581), KX958613, KX959110, KX958861; ***Odontites bocconii***, 625, s, Italy, Sicily, San Martino delle Scale, 13.2581, 38.0861, *G. Domina, s.n.* (PAL 90581), KX958614, KX959111, KX958862; ***Odontites bocconii***, 694, h, Italy, Sicily, Chiusa Sclafani, 13.28698, 37.66516, *G. Certa, Soc. Éch. Pl. Vasc. Eur. Occid. Médit. nr. 18421* (SALA 118685), KX958681, KX959178, KX958929; ***Odontites bocconii***, 823, s, Italy, Sicily, Le Madonie National Park, near Monte Scalone, 14.02147, 37.84662, *J. Peñas de Giles & al., JPG-11-03* (SALA 142125), KX958710, KX959207, KX958958; ***Odontites bocconii***, 824, s, Italy, Sicily, Le Madonie National Park, near Monte Scalone, 14.02147, 37.84662, *J. Peñas de Giles & al., JPG-11-03* (SALA 142125), KX958711, KX959208, KX958959; ***Odontites bocconii***, O.13, h, Italy, Sicily, Chiusa Sclafani, 13.28698, 37.66516, *G. Certa, Soc. Éch. Pl. Vasc. Eur. Occid. Médit. nr. 18421* (M), KX958730, KX959227, KX958978; ***Odontites bocconii***, O.20, h, Italy, Sicily, 9 km N of Polizzi Generosa, 14.01, 37.84, *J.R. Akeroyd & al., 3664* (B 10 0050066), KX958736, KX959233, KX958984; ***Odontites bolligeri*** E.Rico, L.Delgado & Herrero, 89, s, Spain, Granada, Restábal, –3.57582, 36.92045, *M. Martínez Ortega & al., MO 4566* (SALA 135619), KX958517, KX959014, KX958765; ***Odontites bolligeri***, 90, s, Spain, Granada, Restábal, –3.57582, 36.92045, *M. Martínez Ortega & al., MO 4566* (SALA 135619), KX958518, KX959015, KX958766; ***Odontites bolligeri***,

574, s, Morocco, Oriental, Béni Snassen Natural Park, –2.37950, 34.80917, *A. Quintanar & al., AQ 2812* (SALA 142142), KX958563, KX959060, KX958811; ***Odontites bolligeri***, 575, s, Morocco, Oriental, Béni Snassen Natural Park, –2.37950, 34.80917, *A. Quintanar & al., AQ 2812* (SALA 142142), KX958564, KX959061, KX958812; ***Odontites bolligeri***, 609, s, Spain, Málaga, Frigiliana, –3.89672, 36.77680, *D. Pinto Carrasco & V. Lucía, DP 832* (SALA 136804), KX958598, KX959095, KX958846; ***Odontites bolligeri***, 610, s, Spain, Málaga, Frigiliana, –3.89672, 36.77680, *D. Pinto Carrasco & V. Lucía, DP 832* (SALA 136804), KX958599, KX959096, KX958847; ***Odontites bolligeri***, O.17, h, Spain, Almeria, Laujar de Andarax, –2.87629, 37.00067, *L. Posadas & al., s.n.* (MA 861304), KX958733, KX959230, KX958981; ***Odontites bolligeri***, O.18, h, Morocco, Oriental, Béni Snassen Natural Park, –2.37500, 34.81983, *A. Quintanar & al., AQ 2816* (MA 782726), KX958734, KX959231, KX958982; ***Odontites bolligeri***, O.19, h, Morocco, Oriental, Béni Snassen Natural Park, –2.37950, 34.80917, *A. Quintanar & al., AQ 2812* (MA 782731), KX958735, KX959232, KX958983; ***Odontites bolligeri***, O.27, h, Spain, Granada, Orjiva, Sierra de Lújar, –3.4, 36.9*, *H. Merxmüller & W. Gleißner, 29171* (M), KX958743, KX959240, KX958991; ***Odontites cebennensis*** H.J.Coste & Soulié, 87, s, Spain, Barcelona, La Pobla de Lillet, 2.01349, 42.24492, *D. Pinto Carrasco & al., DP 628* (SALA 135679), KX958515, KX959012, KX958763; ***Odontites cebennensis***, 88, s, Spain, Barcelona, La Pobla de Lillet, 2.01349, 42.24492, *D. Pinto Carrasco & al., DP 628* (SALA 135679), KX958516, KX959013, KX958764; ***Odontites cebennensis***, 607, s, Spain, Barcelona, Cercs, 1.86497, 42.14434, *D. Pinto Carrasco & al., DP 632* (SALA 135683), KX958596, KX959093, KX958844; ***Odontites cebennensis***, 608, s, Spain, Barcelona, Cercs, 1.86497, 42.14434, *D. Pinto Carrasco & al., DP 632* (SALA 135683), KX958597, KX959094, KX958845; ***Odontites cebennensis***, O.03, h, Spain, Barcelona, between Sant Quirze de Besora and Ripoll, 2.2, 42.1*, *M. Bolliger, 0-23* (M), KX958722, KX959219, KX958970; ***Odontites cebennensis***, O.06, h, Spain, Gerona, between Campdevanol and La Pobla de Lillet, 2.1, 42.2*, *M. Bolliger, 0-45* (M), KX958724, KX959221, KX958972; ***Odontites cebennensis***, O.25, h, Spain, Gerona, Ripoll, 2.2, 42.2*, *F. Sennen, s.n.* (M), KX958741, KX959238, KX958989; ***Odontites corsicus*** (Loisel.) G.Don., 564, s, France, Corsica, Olmeta-di-Capocorso, Bocca di San Ghjacintu, 9.3842, 42.7489, *A. Tribsch, s.n.* (SALA 137639), KX958553, KX959050, KX958801; ***Odontites corsicus***, 565, s, France, Corsica, Olmeta-di-Capocorso, Bocca di San Ghjacintu, 9.3842, 42.7489, *A. Tribsch, s.n.* (SALA 137639), KX958554, KX959051, KX958802; ***Odontites corsicus***, 819, s, France, Corsica, Olmeta-di-Capocorso, Bocca di San Ghjacintu, 9.3842, 42.7489, *A. Tribsch, s.n.* (SALA 137639), KX958706, KX959203, KX958954; ***Odontites corsicus***, 820, s, France, Corsica, Olmeta-di-Capocorso, Bocca di San Ghjacintu, 9.3842, 42.7489, *A. Tribsch, s.n.* (SALA 137639), KX958707, KX959204, KX958955; ***Odontites corsicus***, O.14, h, France, Corsica, Col de Vergio, 8.9, 42.3*, *J. Lambinon, 98/765 Soc. Éch. Pl. Vasc. Eur. Occid. Médit. nr. 19537* (M), KX958731, KX959228, KX958979; ***Odontites cyprius*** Boiss. [***Odontites linkii*** subsp. ***cyprius*** (Boiss.) Bolliger], 695, h, Cyprus, Larnaka, Páno Léfkara, 33.3, 34.9*, *Alziar & al., Iter Med. IV nr. 0762* (MA 495442), KX958682, KX959179, KX958930; ***Odontites cyprius*** [***Odontites linkii*** subsp. ***cyprius***], O.22, h, Cyprus, Kirenia, Saint Hilarion, 33.2547, 35.3078, *E. Vitek, 04-1961* (M), KX958738, KX959235, KX958986; ***Odontites foliosus*** Peréz Lara, 91, s, Spain, Cádiz, Puerto Real, –6.14, 36.51, *E. Rico & al., ER 7939* (SALA 134536), KX958519, KX959016, KX958767; ***Odontites foliosus***, 92, s, Spain, Cádiz, Puerto Real, –6.14, 36.51, *E. Rico & al., ER 7939* (SALA 134536), KX958520, KX959017, KX958768; ***Odontites foliosus***, 611, s, Spain, Málaga, Manilva, –5.26139, 36.34094, *D. Pinto Carrasco & al., DP 821* (SALA 156297), KX958600, KX959097, KX958848; ***Odontites foliosus***, 612, s, Spain, Málaga, Manilva, –5.26139, 36.34094, *D. Pinto Carrasco & al., DP 821* (SALA 156297), KX958601, KX959098, KX958849; ***Odontites foliosus***, 626, s, Spain, Cádiz, Barbate, Los Caños de Meca, –5.958, 36.182, *E. Rico, ER 7903* (**SALA 103775), KX958615, KX959112, KX958863; ***Odontites glutinosus*** (M.Bieb.) Benth. [***Macrosyringion glutinosum*** (M.Bieb.) Rothm.], 650, h, Bulgaria, Pernik, Golo Bŭrdo, 23.1, 42.6*, *H. Kocev & N. Vihodcevsky, Pl. Bu. Ex., Cent. VI nr.586* (MA 183144), KX958639, KX959136, KX958887; ***Odontites glutinosus*** [***Macrosyringion glutinosum***], 651, h, Turkey, Erzincan, Sakaltutan Geçidi, 39.12, 39.87, *J. Aldasoro & al., A-2687* (MA 689821), KX958640, KX959137, KX958888; ***Odontites hollianus*** (Lowe) Benth., 560, s, Portugal, Madeira, between O Ninho da Manta and O Pico Cidrão, –16.94415, 32.74116, *M. Sequeira & al., MS 5056* (SALA 125030), KX958549, KX959046, KX958797; ***Odontites hollianus***, 561, s, Portugal, Madeira, between O Ninho da Manta and O Pico Cidrão, –16.94415, 32.74116, *M. Sequeira & al., MS 5056* (SALA 125030), KX958550, KX959047, KX958798; ***Odontites hollianus***, 562, s, Spain, Isla de La Palma, Tijarafe, –17.92329, 28.73860, *M. Díaz & al., PALMA 240610* (SALA 156496), KX958551, KX959048, KX958799; ***Odontites hollianus***, 563, s, Spain, Isla de La Palma, Tijarafe, –17.92329, 28.73860, *M. Díaz & al., PALMA 240610* (SALA 156496), KX958552, KX959049, KX958800; ***Odontites hollianus***, 616, s, Portugal, Madeira, between Pico do Arieiro and Pico Ruivo, –16.94, 32.73, *S. Castroviejo & al., SC 17379* (MA 714540), KX958605, KX959102, KX958853; ***Odontites hollianus***, 617, s, Spain, Isla de La Palma, Tijarafe, –17.92329, 28.73860, *M. Díaz & al., PALMA 240610* (SALA 156496), KX958606, KX959103, KX958854; ***Odontites hollianus***, 618, s, Spain, Isla de La Palma, Tijarafe, –17.92329, 28.73860, *M. Díaz & al., PALMA 240610* (SALA 156496), KX958607, KX959104, KX958855; ***Odontites hollianus***, 652, h, Portugal, Madeira, Encumeada, –17.0, 32.8*, *M. Velayos, 9717* (MA 655312), KX958641, KX959138, KX958889; ***Odontites hollianus***, 653, h, Portugal, Madeira, between Ribeiro Frio and Os Balcoes, –16.9, 32.7*, *J. Malato Beliz, 1698* (MA 303197), KX958642, KX959139, KX958890; ***Odontites jaubertianus*** (Boreau) D.Dietr., O.09, h, France, Charente, Saint-Angeau, 0.3, 45.8*, *A. Terrisse, 0-151* (M), KX958726, KX959223, KX958974; ***Odontites kaliformis*** (Pourr. ex Willd.) Pau, 5, s, Spain, Valencia, Sagunto, –0.25, 39.63, *E. Rico & al., ER 7913* (SALA 124706), KX958502, KX958999, KX958750; ***Odontites kaliformis***, 6, s, Spain, Valencia, Sagunto, –0.25, 39.63, *E. Rico & al., ER 7913* (SALA 124706), KX958503, KX959000, KX958751; ***Odontites kaliformis***, 8, s, Spain, Castellón, Cabanes, 0.18, 40.18, *E. Rico & al., ER 7914* (SALA 124707), KX958504, KX959001, KX958752; ***Odontites kaliformis***, 10, s, Spain, Castellón, Cabanes, 0.18, 40.18, *E. Rico & al., ER 7914* (SALA 124707), KX958505, KX959002, KX958753; ***Odontites lapiei*** Batt., 696, h, Algeria, Tizi Ouzou, Djurdjura National Park, Djebel Heïdzer, 4.0, 36.5*, *A. Dubuis & al., Soc. Éch. Pl. Vasc. Eur. Occid. Médit. nr. 18423* (SALA 118683), KX958683, KX959180, KX958931; ***Odontites lapiei***, 697, h, Algeria, Tizi Ouzou, Djurdjura National Park, Djebel Heïdzer, 4.0, 36.5*, *A. Dubuis & al., Soc. Éch. Pl. Vasc. Eur. Occid. Médit. nr. 18423* (SALA 118683), KX958684, KX959181, KX958932; ***Odontites lapiei***, O.10, h, Algeria, Tizi Ouzou, Djurdjura National Park, Djebel Heïdzer, 4.0, 36.5*, *A. Dubuis & al., Soc. Éch. Pl. Vasc. Eur. Occid. Médit. nr. 18423* (MSB), KX958727, KX959224, KX958975; ***Odontites linkii*** Heldr. & Sart. ex Boiss., 570, s, Greece, Corinthia, Trikala, 22.4639, 37.9832, *A. Herrero & al., AH 3480* (SALA 140486), KX958559, KX959056, KX958807; ***Odontites linkii***, 571, s, Greece, Corinthia, Trikala, 22.4639, 37.9832, *A. Herrero & al., AH 3480* (SALA 140486), KX958560, KX959057, KX958808; ***Odontites linkii***, 621, s, Greece, Achaea, between Kalávrita and Kalivitis, 22.1708, 38.0807, *A. Herrero & al., AH 3359* (SALA 140386), KX958610, KX959107, KX958858; ***Odontites linkii***, 622, s, Greece, Lakonia, Lagadha gorge, 22.3355, 37.0940, *C. Aedo, CA 14257* (SALA 140800), KX958611, KX959108, KX958859; ***Odontites linkii***, 623, s, Greece, Lakonia, Lagadha gorge, 22.3355, 37.0940, *C. Aedo, CA 14257* (SALA 140800), KX958612, KX959109, KX958860; ***Odontites longiflorus*** (Lam.) G.Don [***Macrosyringion longiflorum*** (Lam.) Rothm.], 82, s, Spain, Burgos, Castrillo de la Vega, –3.77922, 41.62688, *D. Pinto Carrasco, DP 11* (SALA 135639), KX958510, KX959007, KX958758; ***Odontites longiflorus*** [***Macrosyringion longiflorum***], 586, s, Morocco, Tanger-Tétouan, Jbel L'akraa, –5.1423, 35.1366, *V. Lucía & al., VL 82* (SALA 137638), KX958575, KX959072, KX958823; ***Odontites longiflorus*** [***Macrosyringion longiflorum***], 587, s, Morocco, Tanger-Tétouan, Jbel L'akraa, –5.1423, 35.1366, *V. Lucía & al., VL 82* (SALA 137638), KX958576, KX959073, KX958824; ***Odontites longiflorus*** [***Macrosyringion longiflorum***], 588, s, Spain, Soria, Aldehuela de Periañez, –2.34517, 41.81283, *D. Pinto Carrasco, DP 851* (SALA 137313), KX958577, KX959074, KX958825; ***Odontites longiflorus*** [***Macrosyringion longiflorum***], 589, s, Spain, Soria, Aldehuela de Periañez, –2.34517, 41.81283, *D. Pinto Carrasco, DP 851* (SALA 137313), KX958578, KX959075, KX958826; ***Odontites longiflorus*** [***Macrosyringion longiflorum***], 590, s, Spain, Segovia, Ayllón, Grado del Pico, –3.23325, 41.31703, *D. Pinto Carrasco, DP 898* (SALA 137290), KX958579, KX959076, KX958827; ***Odontites longiflorus*** [***Macrosyringion longiflorum***], 591, s, Spain, Segovia, Ayllón, Grado del Pico, –3.23325, 41.31703, *D. Pinto Carrasco, DP 898* (SALA 137290), KX958580, KX959077, KX958828; ***Odontites longiflorus*** [***Odontites longiflorus*** var. ***roseus*** A.Segura], 647, h, Spain, Soria, between Renieblas and Torretartajo, –2.4, 41.8*, *A. Segura Zubizarreta, 10421, Soc. Éch. Pl. Vasc. Eur. Occid. Médit. nr. 9646* (SALA 63086), KX958636, KX959133, KX958884; ***Odontites longiflorus*** [***Odontites longiflorus*** var. ***roseus***], 648, h, Spain, Soria, between Renieblas and Torretartajo, –2.4, 41.8*, *A. Segura Zubizarreta, 10421, Soc. Éch. Pl. Vasc. Eur.*

Occid. Médit. nr. 9646 (SALA 63086), KX958637, KX959134, KX958885; ***Odontites longiflorus*** [***Odontites longiflorus*** subsp. ***lateritius*** (Charpin & Fern.Casas) Sánchez-Gómez], 649, h, Spain, Murcia, Moratalla, pico Revolcadores, –2.27, 38.07, *A. Aparicio & al., 8807* (MA 594052), KX958638, KX959135, KX958886; ***Odontites luteus*** subsp. ***lanceolatus*** (Gaudin) P.Fourn. [***Odontites lanceolatus*** (Gaudin) Rchb. subsp. ***lanceolatus***], 677, h, France, Hautes-Alpes, Saint-Crépin, 6.6, 44.7*, *G. Dudartre, Soc. Éch. Pl. Vasc. Eur. Occid. Médit. nr. 18422* (SALA 118684), KX958665, KX959162, KX958913; ***Odontites luteus*** subsp. ***lanceolatus*** [***Odontites lanceolatus*** subsp. ***lanceolatus***], 678, h, Italy, Piedmont, between Col de Tende and Coni, 7.6, 44.2*, *M. Bolliger, 0-72* (MA 538540), KX958666, KX959163, KX958914; ***Odontites luteus*** subsp. ***lanceolatus*** [***Odontites lanceolatus*** subsp. ***lanceolatus***], 679, h, France, Hautes-Alpes, Col du Lautaret, 6.3, 45.0*, *M. Bolliger & R. Bolliger, 0-16* (MA 538542), KX958667, KX959164, KX958915; ***Odontites luteus*** subsp. ***lanceolatus*** [***Odontites lanceolatus*** subsp. ***lanceolatus***], O.26, h, France, Isère, Les Ougiers, 6.1, 45.0*, *O. Angerer, s.n.* (M), KX958742, KX959239, KX958990; ***Odontites luteus*** (L.) Clairv. subsp. ***luteus***, 83, s, Spain, Lerida, between Puente de Montañana and Tremp, 0.77634, 42.16084, *E. Rico, ER 7852* (SALA 136275), KX958511, KX959008, KX958759; ***Odontites luteus*** subsp. ***luteus***, 84, s, Spain, Lerida, between Puente de Montañana and Tremp, 0.77634, 42.16084, *E. Rico, ER 7852* (SALA 136275), KX958512, KX959009, KX958760; ***Odontites luteus*** subsp. ***luteus***, 566, s, France, Corsica, Olmeta-di-Capocorso, between Celle and Bocca di San Ghjacintu, 9.3822, 42.7544, *A. Tribsch, s.n.* (SALA 137640), KX958555, KX959052, KX958803; ***Odontites luteus*** subsp. ***luteus***, 567, s, France, Corsica, Olmeta-di-Capocorso, between Celle and Bocca di San Ghjacintu, 9.3822, 42.7544, *A. Tribsch, s.n.* (SALA 137640), KX958556, KX959053, KX958804; ***Odontites luteus*** subsp. ***luteus***, 568, s, Croatia, Splitsko-Dalmatinska, between Brela and Gornja Brela, 16.89363, 43.40381, *M. Martínez Ortega & al., MO 5538* (SALA 137346), KX958557, KX959054, KX958805; ***Odontites luteus*** subsp. ***luteus***, 569, s, Croatia, Splitsko-Dalmatinska, between Brela and Gornja Brela, 16.89363, 43.40381, *M. Martínez Ortega & al., MO 5538* (SALA 137346), KX958558, KX959055, KX958806; ***Odontites luteus*** subsp. ***luteus***, 602, s, Spain, Teruel, Arbolí, 0.94110, 41.22580, *D. Pinto Carrasco & al., DP 641* (SALA 135692), KX958591, KX959088, KX958839; ***Odontites luteus*** subsp. ***luteus***, 603, s, Spain, Teruel, Arbolí, 0.94110, 41.22580, *D. Pinto Carrasco & al., DP 641* (SALA 135692), KX958592, KX959089, KX958840; ***Odontites luteus*** subsp. ***luteus***, 642, s, Spain, Albacete, Riópar, –2.39010, 38.49901, *D. Pinto Carrasco & al., DP 763* (SALA 137330), KX958631, KX959128, KX958879; ***Odontites luteus*** subsp. ***luteus***, 643, s, Spain, Albacete, Riópar, –2.39010, 38.49901, *D. Pinto Carrasco & al., DP 763* (SALA 137330), KX958632, KX959129, KX958880; ***Odontites luteus*** subsp. ***luteus***, 658, h, France, Var, between Riez and Sainte-Croix-du-Verdon, 6.1, 43.8*, *M. Bolliger, 0-60* (MA 538314), KX958647, KX959144, KX958895; ***Odontites luteus*** subsp. ***luteus***, 659, h, France, Var, between Moustiers-Sainte-Marie and La Palud-sur-Verdon, 6.2, 43.8*, *M. Bolliger, 0-59* (MA 538312), KX958648, KX959145, KX958896; ***Odontites luteus*** subsp. ***luteus***, 661, h, France, Var, La Garde-Freinet, 6.5, 43.3*, *M. Bolliger & R. Bolliger, 0-145* (MA 538310), KX958649, KX959146, KX958897; ***Odontites luteus*** subsp. ***luteus***, 662, h, France, Alpes-Maritimes, Roussillon, 5.3, 43.9*, *M. Bolliger, 0-68* (MA 538307), KX958650, KX959147, KX958898; ***Odontites luteus*** subsp. ***luteus***, 663, h, France, Bouches-du-Rhône, Allauch, 5.5, 43.3*, *P. Martin, Soc. Éch. Pl. Vasc. Eur. Occid. Médit. nr. 6868* (MA 303024), KX958651, KX959148, KX958899; ***Odontites luteus*** subsp. ***luteus***, 664, h, Italy, Sardinia, Badesi, 8.9114, 41.0306, *C. Aedo & al., 9108* (MA 708621), KX958652, KX959149, KX958900; ***Odontites luteus*** subsp. ***luteus***, 665, h, France, Corrèze, between Noailles and Cressensac, 1.5, 45.1*, *M. Bolliger & R. Bolliger, 0-7* (MA 538313), KX958653, KX959150, KX958901; ***Odontites luteus*** subsp. ***luteus***, 666, h, Italy, Umbria, Norcia, 13.1, 42.8*, *A. Pavesi, Soc. Éch. Pl. Vasc. Eur. Occid. Médit. nr. 13551* (SALA 82871), KX958654, KX959151, KX958902; ***Odontites luteus*** subsp. ***luteus***, 667, h, Germany, Bavaria, Landkreis Neumarkt, Buchenberg, 11.7, 49.2*, *N. Meyer, Soc. Éch. Pl. Vasc. Eur. Occid. Médit. nr. 19539* (SALA 119350), KX958655, KX959152, KX958903; ***Odontites luteus*** subsp. ***luteus***, 829, s, Spain, Valladolid, Santibañez de Valcorba, –4.44673, 41.57944, *D. Pinto Carrasco, DP 1018* (SALA 110042), KX958716, KX959213, KX958964; ***Odontites luteus*** subsp. ***luteus***, 830, s, Spain, Valladolid, Santibañez de Valcorba, –4.44673, 41.57944, *D. Pinto Carrasco, DP 1018* (SALA 110042), KX958717, KX959214, KX958965; ***Odontites luteus*** subsp. ***luteus***, 831, s, Czech Republic, South Moravia, between Klentnice and Mikulov, 16.64113, 48.83663, *B. Rojas-Andrés & al., BR 187* (SALA 142123), KX958718, KX959215, KX958966; ***Odontites luteus*** subsp. ***luteus***, 832, s, Czech Republic, South Moravia, between Klentnice and Mikulov, 16.64113, 48.83663, *B. Rojas-Andrés & al., BR 187* (SALA 142123), KX958719, KX959216, KX958967; ***Odontites luteus*** subsp. ***provincialis*** (Bolliger) J.-M.Tison. [***Odontites lanceolatus*** subsp. ***provincialis*** Bolliger], 680, h, France, Alpes-Maritimes, Col de Ferrier, 6.9, 43.7*, *M. Bolliger & R. Bolliger, 0-13* (MA 538451), KX958668, KX959165, KX958916; ***Odontites luteus*** subsp. ***provincialis*** [***Odontites lanceolatus*** subsp. ***provincialis***], 681, h, France, Var, between Castellane and La Palud-sur-Verdon, 6.4, 43.8*, *M. Bolliger, 0-57* (MA 538452), KX958669, KX959166, KX958917; ***Odontites luteus*** subsp. ***provincialis*** [***Odontites lanceolatus*** subsp. ***provincialis***], 682, h, France, Alpes-Maritimes, La Penne, 6.9, 43.9*, *M. Bolliger, 0-65* (MA 538453), KX958670, KX959167, KX958918; ***Odontites luteus*** subsp. ***provincialis*** [***Odontites lanceolatus*** subsp. ***provincialis***], 683, h, France, Alpes-Maritimes, Col de Braus, 7.4, 43.9*, *M. Bolliger, 0-70* (MA 538459), KX958671, KX959168, KX958919; ***Odontites luteus*** subsp. ***provincialis*** [***Odontites lanceolatus*** subsp. ***provincialis***], O.02, h, France, Alpes-Maritimes, Tinée Valley, 7.2, 43.9*, *M. Bolliger, 0-69* (M), KX958721, KX959218, KX958969; ***Odontites maroccanus*** Bolliger, 578, s, Morocco, Meknès-Tafilalet, Tizi-n-Tretten, –5.03801, 33.45669, *D. Pinto Carrasco & al., DP 785* (SALA 156299), KX958567, KX959064, KX958815; ***Odontites maroccanus***, 579, s, Morocco, Meknès-Tafilalet, Tizi-n-Tretten, –5.03801, 33.45669, *D. Pinto Carrasco & al., DP 785* (SALA 156299), KX958568, KX959065, KX958816; ***Odontites maroccanus***, 700, h, Morocco, Meknès-Tafilalet, Ifrane, Oued Tizguit, –5.1167, 33.5528, *Crespo & al., s.n.* (ABH 33424), KX958687, KX959184, KX958935; ***Odontites maroccanus***, 701, h, Morocco, Meknès-Tafilalet, Ifrane, Oued Tizguit, –5.1167, 33.5528, *Crespo & al., s.n.* (ABH 33424), KX958688, KX959185, KX958936; ***Odontites maroccanus***, 727, h, Morocco, Meknès-Tafilalet, near Jebel Hebri, –5.15, 33.36, *M.A. Mateos & al., 4396/94* (BC 852667), KX958705, KX959202, KX958953; ***Odontites maroccanus***, O.33, h, Morocco, Meknès-Tafilalet, Timhadit, –5.1, 33.2*, *E. Jahandiez, 881* (M), KX958748, KX959245, KX958996; ***Odontites powellii*** Maire, 580, s, Morocco, Meknès-Tafilalet, Tizi-n-Tretten, –5.03961, 33.45582, *D. Pinto Carrasco & al., DP 786* (SALA 156298), KX958569, KX959066, KX958817; ***Odontites powellii***, 581, s, Morocco, Meknès-Tafilalet, Tizi-n-Tretten, –5.03961, 33.45582, *D. Pinto Carrasco & al., DP 786* (SALA 156298), KX958570, KX959067, KX958818; ***Odontites powellii***, 582, s, Morocco, Tanger-Tétouan, Jbel L'akraa, –5.14364, 35.13683, *V. Lucía & al., VL 83* (SALA 156300), KX958571, KX959068, KX958819; ***Odontites powellii***, 583, s, Morocco, Tanger-Tétouan, Jbel L'akraa, –5.14364, 35.13683, *V. Lucía & al., VL 83* (SALA 156300), KX958572, KX959069, KX958820; ***Odontites powellii***, 584, s, Morocco, Tadla-Azilal, Tizi Aït Ouirrah, –6.0186, 32.5208, *A. Quintanar & al., AQ 2119* (MA 746128), KX958573, KX959070, KX958821; ***Odontites powellii***, 585, s, Morocco, Tadla-Azilal, Tizi Aït Ouirrah, –6.0186, 32.5208, *A. Quintanar & al., AQ 2119* (MA 746128), KX958574, KX959071, KX958822; ***Odontites powellii***, O.11, h, Morocco, Meknès-Tafilalet, between Ifrane and Zaouïa d'Ifrane, –5.1, 33.6*, *M. Bolliger & D. Moser, 0-163* (M), KX958728, KX959225, KX958976; ***Odontites pyrenaeus*** subsp. ***abilianus*** P.Monts., 606, s, Spain, Huesca, Jaca, Bernués, –0.60, 42.49, *E. Rico, ER 7746* (SALA 103068), KX958595, KX959092, KX958843; ***Odontites pyrenaeus*** subsp. ***abilianus***, 692, h, Spain, Huesca, Jaca, monte de Larbesa, –0.55, 42.54, *G. Montserrat, s.n.* (SALA 23738), KX958679, KX959176, KX958927; ***Odontites pyrenaeus*** subsp. ***abilianus***, 693, h, Spain, Huesca, Jaca, Casa de Valpregona, –0.58, 42.48, *E. Rico, ER 7745* (SALA 103067), KX958680, KX959177, KX958928; ***Odontites pyrenaeus*** (Bubani) Rothm. subsp. ***pyrenaeus***, 85, s, Spain, Lerida, Sarroca de Bellera, 0.86660, 42.36414, *D. Pinto Carrasco & al., DP 615* (SALA 135664), KX958513, KX959010, KX958761; ***Odontites pyrenaeus*** subsp. ***pyrenaeus***, 86, s, Spain, Lerida, Sarroca de Bellera, 0.86660, 42.36414, *D. Pinto Carrasco & al., DP 615* (SALA 135664), KX958514, KX959011, KX958762; ***Odontites pyrenaeus*** subsp. ***pyrenaeus***, 604, s, Spain, Huesca, Plan, Saravillo, 0.26438, 42.55771, *E. Rico, ER 7845* (SALA 136276), KX958593, KX959090, KX958841; ***Odontites pyrenaeus*** subsp. ***pyrenaeus***, 605, s, Spain, Huesca, Plan, Saravillo, 0.26438, 42.55771, *E. Rico, ER 7845* (SALA 136276), KX958594, KX959091, KX958842; ***Odontites pyrenaeus*** subsp. ***pyrenaeus***, O.01, h, Spain, Lerida, between El Pont de Suert and Saroca de Bellera, 0.8, 42.4*, *M. Bolliger, 0-38* (M), KX958720, KX959217, KX958968; ***Odontites rameauanus*** Emb., 576, s, Morocco, Tadla-Azilal, Jebel Tarkeddid, –6.5069, 31.5464, *A. Quintanar & al., AQ 2129* (MA 746138), KX958565, KX959062, KX958813; ***Odontites rameauanus***, 577, s, Morocco, Tadla-Azilal, Jebel Tarkeddid, –6.5069, 31.5464, *A. Quintanar & al., AQ 2129* (MA 746138), KX958566,

KX959063, KX958814; ***Odontites recordonii*** Burnat & Barbey, 138, s, Spain, Alava, Bachicabo, –3.06771, 42.77934, *M. Martínez Ortega, MO 4526* (SALA 135620), KX958525, KX959022, KX958773; ***Odontites recordonii***, 170, s, Spain, Teruel, Formiche Alto, Formiche Bajo, –0.88, 40.29, *L. Delgado Sánchez & al., LD 1020* (SALA 135630), KX958526, KX959023, KX958774; ***Odontites recordonii***, 197, s, Spain, Valencia, El Saler, –0.32096, 39.36067, *D. Pinto Carrasco & al., DP 676* (SALA 135726), KX958527, KX959024, KX958775; ***Odontites recordonii***, 249, s, Spain, Teruel, Arbolí, 0.96434, 41.23127, *D. Pinto Carrasco & al., DP 642* (SALA 135693), KX958528, KX959025, KX958776; ***Odontites recordonii***, 255, s, Spain, Zaragoza, Paracuellos de Jiloca, –1.61225, 41.32468, *D. Pinto Carrasco & al., DP 653* (SALA 135704), KX958529, KX959026, KX958777; ***Odontites recordonii***, 301, s, Spain, Guadalajara, Fuentelviejo, –2.98810, 40.51173, *D. Pinto Carrasco & al., DP 692* (SALA 135742), KX958530, KX959027, KX958778; ***Odontites recordonii***, O.07, h, Spain, Zaragoza, between Urriés and Ruesta, –1.1, 42.6*, *M. Bolliger, 0-30 (M),* KX958725, KX959222, KX958973; ***Odontites sillettii*** Brullo, Tomaselli & Wagens., O.34, h, Italy, Apulia, Santeramo in Colle, 16.8, 40.8*, *G.N. Silletti & V. Tomaselli, s.n.* (MSB), KX958749, KX959246, KX958997; ***Odontites vernus*** (Bellardi) Dumort., 668, h, Denmark, Zealand, Hundige, 12.3, 55.6*, *A. Hansen, Soc. Éch. Pl. Vasc. Eur. Occid. Médit. nr. 13552* (SALA 82870), KX958656, KX959153, KX958904; ***Odontites vernus***, 669, h, Germany, Schleswig-Holstein, Leck, Büllsbüll, 9.0, 54.8*, *P. Pedersen & al., Fl. Ger. Ex., Schl.-Hols. nr. 166* (MA 327862), KX958657, KX959154, KX958905; ***Odontites vernus***, 670, h, Denmark, Island Fur in Limfjorden, Faerker Hede, 9.0, 56.8*, *K. Larsen & S.S. Larsen, 40738, Soc. Éch. Pl. Vasc. Eur. Occid. Médit. nr. 15514* (MA 532164), KX958658, KX959155, KX958906; ***Odontites vernus***, 671, h, Russia, Moscow, Abramtsevo, 38.0, 56.2*, *E.E. Gogina, s.n.* (MA 303021), KX958659, KX959156, KX958907; ***Odontites vernus***, 672, h, Belgium, Wallonia, Tihange (Huy), 5.3, 50.5*, *M. Reekmans, 11345, Soc. Éch. Pl. Vasc. Eur. Occid. Médit. nr. 11665* (SALA 75333), KX958660, KX959157, KX958908; ***Odontites vernus***, 673, h, France, Bas-Rhin, Auenheim, 8.0, 48.8*, *A. Schneider, Soc. Éch. Pl. Vasc. Eur. Occid. Médit. nr. 9650* (SALA 63091), KX958661, KX959158, KX958909; ***Odontites vernus***, 674, h, France, motorway Nîmes-Lyon km 111, 4.8, 44.6*, *unknown, 733PV* (MA 443277), KX958662, KX959159, KX958910; ***Odontites vernus***, 675, h, Italy, Veneto, Caorle, 12.9, 45.6*, *F. Cernoch, 53749 FC, Soc. Éch. Pl. Vasc. Eur. Occid. Médit. nr. 16440* (SALA 88671), KX958663, KX959160, KX958911; ***Odontites vernus***, 676, h, Great Britain, Hampshire, Winchester, near Itchen Down Farm, –1.2, 51.1*, *S.L. Lury, Soc. Éch. Pl. Vasc. Eur. Occid. Médit. nr. 15519* (SALA 88476), KX958664, KX959161, KX958912; ***Odontites vernus***, 716, h, Russia, Altai Krai, Kadnikovo, 81.88878, 52.87285, *S.I. Molokanov & A.P. Schalimov, s.n.* (ALTB), KX958699, KX959196, KX958947; ***Odontites vernus***, 717, h, Russia, Khakassia, foot of a ridge Alan, 91.08460, 52.80173, *A.P. Shalimov & A.D. Djagilev, s.n.* (ALTB), KX958700, KX959197, KX958948; ***Odontites vernus***, 718, h, Russia, Altai Krai, Mikhailovka, east coast of lake Kulundinskoe, 79.8, 53.0*, *D.A. German & D.A. Durnikin, s.n.* (ALTB), KX958701, KX959198, KX958949; ***Odontites vernus***, 719, h, Russia, Altai Republic, near settlement Aktal, 88.87, 49.92, *R.V. Kamelin & al., ADSCH 2328* (ALTB), KX958702, KX959199, KX958950; ***Odontites vernus***, 720, h, Mongolia, Kobdosskii, 10 km at South from Bulgan, 91.5, 46.0*, *D.L. Belkin & A.P. Shalimov, s.n.* (ALTB), KX958703, KX959200, KX958951; ***Odontites vernus***, 721, h, Russia, Altai Krai, between Gonokhovo and Dobraya Volya, 81.24068, 52.92590, *A.I. Shmakov & al., s.n.* (ALTB), KX958704, KX959201, KX958952; ***Odontites vernus***, O.12, h, Germany, Bavaria, Neustadt an der Waldnaab, 12.2, 49.7*, *W. Lippert, 27604* (M), KX958729, KX959226, KX958977; ***Odontites vernus***, O.29, h, USA, Maine, Aroostook County, –68.3397, 46.1247, *D. Atha & M. Lee, 2235* (M), KX958744, KX959241, KX958992; ***Odontites vernus***, O.30, h, China, Xinjiang, Atojnagh Valley, 75.17, 38.95, *U. Wündisch, 748* (MSB 147298), KX958745, KX959242, KX958993; ***Odontites vernus***, O.31, h, Finland, Hämeenlinna, 750m SW of Vanaja church, 24.5, 61.0*, *R. Lampinen, 10379* (M), KX958746, KX959243, KX958994; ***Odontites vernus***, O.32, h, Germany, Bavaria, Landkreis Freising, Eching, 11.6, 48.3*, *Plachter, s.n.* (M 0248419), KX958747, KX959244, KX958995; ***Odontites vernus*** subsp. ***fennicus*** (Markl.) Pinto-Carrasco, E.Rico & M.M.Mart.Ort. [***Odontites litoralis*** subsp. ***fennicus*** Markl.], 688, h, Finland, Uusimaa, Porvoon mlk, Sundby, Karilamalm, 25.87, 60.27, *U. Laine & al., s.n.* (SALA 50565), KX958675, KX959172, KX958923; ***Odontites vernus*** subsp. ***fennicus*** [***Odontites litoralis*** subsp. ***fennicus***], 689, h, Finland, Uusimaa, Ruotsinpyhtää, Söderby, Ropanudden, 26.53, 60.33, *U. Laine & al., s.n.* (SALA 50566), KX958676, KX959173, KX958924; ***Odontites vernus*** subsp. ***fennicus*** [***Odontites litoralis*** subsp. ***fennicus***], 690, h, Finland, Uusimaa, Storpellinge island, istmus between Koräs and Cape Sandholmen, 25.9, 60.2*, *R. Lampinen, 9302, Soc. Éch. Pl. Vasc. Eur. Occid. Médit. nr. 15511* (SALA 88468), KX958677, KX959174, KX958925; ***Odontites vernus*** subsp. ***fennicus*** [***Odontites litoralis*** subsp. ***fennicus***], 691, h, Finland, Varsinais-Suomi, Nauvo, Västergård, 22.0, 60.2*, *R. Murto & M. Koistinen, 3833, Soc. Éch. Pl. Vasc. Eur. Occid. Médit. nr. 15515* (SALA 88472), KX958678, KX959175, KX958926; ***Odontites vernus*** subsp. ***himalayicus*** (Pennell) Pinto-Carrasco, E.Rico & M.M.Mart.Ort. [***Odontites vulgaris*** subsp. ***himalayicus*** (Pennell) Bolliger], O.23, h, Pakistan, Kashmir, Hushe River, 76.4, 35.3*, *G.L. Webster & E. Nasir, 6290* (M), KX958739, KX959236, KX958987; ***Odontites vernus*** subsp. ***litoralis*** (Fr.) Nyman [***Odontites litoralis*** (Fr.) Fr. subsp. ***litoralis***], 685, h, Finland, Varsinais-Suomi, Lokalahti, 21.27, 60.72, *S. Hinneri, s.n.* (SALA 29309), KX958672, KX959169, KX958920; ***Odontites vernus*** subsp. ***litoralis*** [***Odontites litoralis*** subsp. ***litoralis***], 686, h, Finland, Varsinais-Suomi, Jungfruskär island, 21.08, 60.13, *K. Alho & al., s.n.* (SALA 50567), KX958673, KX959170, KX958921; ***Odontites vernus*** subsp. ***litoralis*** [***Odontites litoralis*** subsp. ***litoralis***], 687, h, Finland, Varsinais-Suomi, Korppoo, Kälö Island, 21.38, 60.08, *U. Laine & al., s.n.* (SALA 50445), KX958674, KX959171, KX958922; ***Odontites vernus*** subsp. ***serotinus*** Corb. [***Odontites vulgaris*** Moench. subsp. ***vulgaris***], 48, s, Spain, Valladolid, Aldeamayor de San Martín, –4.61, 41.52, *L. Delgado Sánchez & M. Santos Vicente, LD 908* (**SALA 110700), KX958506, KX959003, KX958754; ***Odontites vernus*** subsp. ***serotinus*** [***Odontites vulgaris*** subsp. ***vulgaris***], 57, s, Spain, Lugo, Samos, Renche, –7.22, 42.72, *E. Rico, ER 7890* (SALA 110730), KX958508, KX959005, KX958756; ***Odontites vernus*** subsp. ***serotinus*** [***Odontites vulgaris*** subsp. ***vulgaris***], 339, s, Greece, Arcadia, between Trípoli and Nestáni, 22.4236, 37.5834, *L. Medina & al., LM 4132* (MA 762104), KX958531, KX959028, KX958779; ***Odontites vernus*** subsp. ***serotinus*** [***Odontites vulgaris*** subsp. ***vulgaris***], 340, s, Greece, Arcadia, between Trípoli and Nestáni, 22.4236, 37.5834, *L. Medina & al., LM 4132* (MA 762104), KX958532, KX959029, KX958780; ***Odontites vernus*** subsp. ***serotinus*** [***Odontites vulgaris*** subsp. ***vulgaris***], 440, s, Turkey, Kırklareli, Dereköy, 27.35305, 41.93761, *B. Rojas-Andrés & al., BR 39* (SALA 135613), KX958535, KX959032, KX958783; ***Odontites vernus*** subsp. ***serotinus*** [***Odontites vulgaris*** subsp. ***vulgaris***], 463, s, Spain, Granada, Quéntar, –3.40546, 37.23347, *D. Pinto Carrasco & al., DP 663* (SALA 135713), KX958536, KX959033, KX958784; ***Odontites vernus*** subsp. ***serotinus*** [***Odontites vulgaris*** subsp. ***vulgaris***], 464, s, Spain, Granada, Quéntar, –3.40546, 37.23347, *D. Pinto Carrasco & al., DP 663* (SALA 135713), KX958537, KX959034, KX958785; ***Odontites vernus*** subsp. ***serotinus*** [***Odontites vulgaris*** subsp. ***vulgaris***], 619, s, Ireland, County Longford, Cashel Nature Reserve, opposite to Cow Island, –7.98694, 53.58889, *D. Doogue, s.n.* (no voucher), KX958608, KX959105, KX958856; ***Odontites vernus*** subsp. ***serotinus*** [***Odontites vulgaris*** subsp. ***vulgaris***], 620, s, Ireland, County Down, North of the lighthouse at St. John's Point, –5.65722, 54.22944, *D. Doogue, s.n.* (no voucher), KX958609, KX959106, KX958857; ***Odontites vernus*** subsp. ***serotinus*** [***Odontites vulgaris*** subsp. ***vulgaris***], 710, s, France, Aisne, Beaurieux, 3.74058, 49.39850, *M. Martínez Ortega & al., MO 6024* (SALA 142126), KX958693, KX959190, KX958941; ***Odontites vernus*** subsp. ***serotinus*** [***Odontites vulgaris*** subsp. ***vulgaris***], 711, s, France, Aisne, Beaurieux, 3.74058, 49.39850, *M. Martínez Ortega & al., MO 6024* (SALA 142126), KX958694, KX959191, KX958942; ***Odontites vernus*** subsp. ***serotinus*** [***Odontites vulgaris*** subsp. ***vulgaris***], 712, s, Germany, Rheinland-Pfalz, near Landau, 8.13750, 49.17658, *M. Martínez Ortega & al., MO 6027* (SALA 142127), KX958695, KX959192, KX958943; ***Odontites vernus*** subsp. ***serotinus*** [***Odontites vulgaris*** subsp. ***vulgaris***], 713, s, Germany, Rheinland-Pfalz, near Landau, 8.13750, 49.17658, *M. Martínez Ortega & al., MO 6027* (SALA 142127), KX958696, KX959193, KX958944; ***Odontites vernus*** subsp. ***serotinus*** [***Odontites vulgaris*** subsp. ***vulgaris***], 714, s, France, Haut-Rhin, Bergheim, 7.35202, 48.21186, *M. Martínez Ortega & al., MO 6031* (SALA 142128), KX958697, KX959194, KX958945; ***Odontites vernus*** subsp. ***serotinus*** [***Odontites vulgaris*** subsp. ***vulgaris***], 715, s, France, Haut-Rhin, Bergheim, 7.35202, 48.21186, *M. Martínez Ortega & al., MO 6031* (SALA 142128), KX958698, KX959195, KX958946; ***Odontites vernus*** subsp. ***siculus*** (Guss.) Sell [***Odontites vulgaris*** subsp. ***siculus*** (Guss.) Bolliger], 348, s, Italy, Sicily, Geraci Siculo, 14.1539, 37.8803, *G. Domina, s.n.* (PAL 88463), KX958533, KX959030, KX958781; ***Odontites vernus*** subsp. ***siculus*** [***Odontites vulgaris*** subsp. ***siculus***], 349, s, Italy, Sicily, Geraci Siculo, 14.1539, 37.8803, *G. Domina, s.n.* (PAL 88463), KX958534, KX959031, KX958782; ***Odontites vernus*** (Bellardi) Dumort. subsp. ***vernus***, 49, s, Spain, Segovia, Valle de Tabladillo, –3.84, 41.36, *L.*

Delgado Sánchez & M. Santos Vicente, LD 976 (SALA 128800), KX958507, KX959004, KX958755; ***Odontites vernus*** subsp. ***vernus***, 530, s, Croatia, Ličko-Senjska, between Prijeboj and Ličko Petrovo Selo, 15.68401, 44.84572, *M. Martínez Ortega & al., MO 5531* (SALA 137348), KX958538, KX959035, KX958786; ***Odontites vernus*** subsp. ***vernus***, 708, s, France, Eure, Evreux, 1.10769, 49.01655, *B. Rojas-Andrés & al., BR 158* (SALA 142120), KX958691, KX959188, KX958939; ***Odontites vernus*** subsp. ***vernus***, 709, s, France, Eure, Evreux, 1.10769, 49.01655, *B. Rojas-Andrés & al., BR 158* (SALA 142120), KX958692, KX959189, KX958940; ***Odontites viscosus*** subsp. ***asturicus*** M.Laínz, 551, s, Spain, León, Puebla de Lillo, Isoba, –5.36313, 43.09460, *D. Pinto Carrasco & E. Rico, DP 874* (SALA 137373), KX958542, KX959039, KX958790; ***Odontites viscosus*** subsp. ***asturicus***, 598, s, Spain, León, Puebla de Lillo, Isoba, –5.36313, 43.09460, *D. Pinto Carrasco & E. Rico, DP 874* (SALA 137373), KX958587, KX959084, KX958835; ***Odontites viscosus*** subsp. ***asturicus***, 599, s, Spain, León, Puebla de Lillo, Isoba, –5.36313, 43.09460, *D. Pinto Carrasco & E. Rico, DP 874* (SALA 137373), KX958588, KX959085, KX958836; ***Odontites viscosus*** subsp. ***asturicus***, 657, h, Spain, Oviedo, Lena, Tuizia de Arriba, Peña Cerreos, –5.9372, 43.0164, *J. Calvo, JC 4008* (MA 790195), KX958646, KX959143, KX958894; ***Odontites viscosus*** subsp. ***asturicus***, O.21, h, Spain, Oviedo, Lena, east of Peña Vera, –5.9, 43.0*, *H. Merxmüller & W. Lippert, 29754, Herb. Lipp. nr. 15383* (M), KX958737, KX959234, KX958985; ***Odontites viscosus*** subsp. ***australis*** (Boiss.) Jahand. & Maire, 93, s, Spain, Granada, Güéjar Sierra, Canales, –3.47717, 37.15449, *D. Pinto Carrasco & B.N. Ariza, DP 566* (SALA 136267), KX958521, KX959018, KX958769; ***Odontites viscosus*** subsp. ***australis***, 94, s, Spain, Granada, Güéjar Sierra, Canales, –3.47717, 37.15449, *D. Pinto Carrasco & B.N. Ariza, DP 566* (SALA 136267), KX958522, KX959019, KX958770; ***Odontites viscosus*** subsp. ***australis***, 548, s, Morocco, Tanger-Tétouan, Jbel L'akraa, –5.14364, 35.13683, *V. Lucía & al., VL 91* (SALA 156301), KX958539, KX959036, KX958787; ***Odontites viscosus*** subsp. ***australis***, 549, s, Spain, Toledo, Orgaz, between Marjaliza and Arisgotas, –3.92741, 39.58057, *V. Lucía & al., VL 30* (SALA 137339), KX958540, KX959037, KX958788; ***Odontites viscosus*** subsp. ***australis***, 827, s, Spain, Zamora, Corrales del Vino, –5.71, 41.34, *E. Rico, ER 7974* (SALA 135647), KX958714, KX959211, KX958962; ***Odontites viscosus*** subsp. ***australis***, 828, s, Spain, Zamora, Corrales del Vino, –5.71, 41.34, *E. Rico, ER 7974* (SALA 135647), KX958715, KX959212, KX958963; ***Odontites viscosus*** subsp. ***eriopodus*** Litard. & Maire, 656, h, Morocco, Meknès-Tafilalet, between Ifrane and Zaouïa d'Ifrane, –5.1, 33.6*, *M. Bolliger & D. Moser, 0-164* (MA 538475), KX958645, KX959142, KX958893; ***Odontites viscosus*** subsp. ***granatensis*** (Boiss.) Bolliger, 95, s, Spain, Granada, Sierra Nevada, Collado de Las Sabinas, –3.42448, 37.11694, *J. **Peñas de** Giles, ODOGRA-G01* (**SALA 135386), KX958523, KX959020, KX958771; ***Odontites viscosus*** subsp. ***granatensis***, 96, s, Spain, Granada, Sierra Nevada, Collado de Las Sabinas, –3.42448, 37.11694, *J. **Peñas de** Giles, ODOGRA-G01* (**SALA 135386), KX958524, KX959021, KX958772; ***Odontites viscosus*** subsp. ***granatensis***, 558, s, Spain, Granada, Sierra Navada, barranco del Guarnón, –3.36, 37.08, *D. Pinto Carrasco & al., DP 893* (SALA 157687), KX958547, KX959044, KX958795; ***Odontites viscosus*** subsp. ***granatensis***, 559, s, Spain, Granada, Sierra Navada, barranco del Guarnón, –3.36, 37.08, *D. Pinto Carrasco & al., DP 893* (SALA 157688), KX958548, KX959045, KX958796; ***Odontites viscosus*** subsp. ***granatensis***, 572, s, Spain, Granada, Sierra Nevada, barranco de Río Seco, –3.34, 37.03, *J. Fuentes, s.n.* (GDA 61399), KX958561, KX959058, KX958809; ***Odontites viscosus*** subsp. ***granatensis***, 573, s, Spain, Granada, Sierra Nevada, barranco de Río Seco, –3.34, 37.03, *J. Fuentes, s.n.* (GDA 61399), KX958562, KX959059, KX958810; ***Odontites viscosus*** subsp. ***granatensis***, 627, s, Spain, Granada, Sierra Nevada, Cerro Cañadillas, –3.42758, 37.12128, *J. **Peñas de** Giles & J. Lorite, JPG 130* (no voucher), KX958616, KX959113, KX958864; ***Odontites viscosus*** subsp. ***granatensis***, 628, s, Spain, Granada, Sierra Nevada, Cerro Cañadillas, –3.42758, 37.12128, *J. **Peñas de** Giles & J. Lorite, JPG 130* (no voucher), KX958617, KX959114, KX958865; ***Odontites viscosus*** subsp. ***lusitanicus*** Bolliger [***Odontites viscosus*** subsp. ***australis***], 550, s, Portugal, Setúbal, Sesimbra, Cabo Espichel, –9.2094, 38.4141, *M. Santos Vicente & al., MS 959* (SALA 123308), KX958541, KX959038, KX958789; ***Odontites viscosus*** subsp. ***lusitanicus*** [***Odontites viscosus*** subsp. ***australis***], 596, s, Portugal, Setúbal, Sesimbra, Cabo Espichel, –9.1880, 38.4210, *M. Santos Vicente & al., MS 961* (SALA 123309), KX958585, KX959082, KX958833; ***Odontites viscosus*** subsp. ***lusitanicus*** [***Odontites viscosus*** subsp. ***australis***], 597, s, Portugal, Setúbal, Sesimbra, Cabo Espichel, –9.1880, 38.4210, *M. Santos Vicente & al., MS 961* (SALA 123309), KX958586, KX959083, KX958834; ***Odontites viscosus*** (L.) Clairv. subsp. ***viscosus***, 552, s, Spain, Barcelona, Vilada, 1.94764, 42.12980, *D. Pinto Carrasco & al., DP 633* (SALA 135684), KX958543, KX959040, KX958791; ***Odontites viscosus*** subsp. ***viscosus***, 600, s, Spain, Lerida, Sarroca de Bellera, 0.86659, 42.36419, *D. Pinto Carrasco & al., DP 616* (SALA 135665), KX958589, KX959086, KX958837; ***Odontites viscosus*** subsp. ***viscosus***, 601, s, Spain, Lerida, Sarroca de Bellera, 0.86659, 42.36419, *D. Pinto Carrasco & al., DP 616* (SALA 135665), KX958590, KX959087, KX958838; ***Odontites viscosus*** subsp. ***viscosus***, 654, h, France, Hautes-Alpes, Villar-Saint-Pancrace, Le Mélézin, 6.6, 44.9*, *G. Dutartre, Soc. Éch. Pl. Vasc. Eur. Occid. Médit. nr. 18426* (SALA 118680), KX958643, KX959140, KX958891; ***Odontites viscosus*** subsp. ***viscosus*** [***Odontites viscosus*** subsp. ***oscensis*** P.Monts.], 655, h, Spain, Huesca, Broto, Asín de Broto, –0.15, 42.53, *P. Montserrat, 6135/74, Soc. Éch. Pl. Vasc. Eur. Occid. Médit. nr. 9652* (SALA 63094), KX958644, KX959141, KX958892; ***Odontites viscosus*** subsp. ***viscosus***, 825, s, France, Bouches-du-Rhône, Palama, 5.43910, 43.37670, *B. Rojas-Andrés & al., BR 165* (SALA 142122), KX958712, KX959209, KX958960; ***Odontites viscosus*** subsp. ***viscosus***, 826, s, France, Bouches-du-Rhône, Palama, 5.43910, 43.37670, *B. Rojas-Andrés & al., BR 165* (SALA 142122), KX958713, KX959210, KX958961; ***Odontites viscosus*** subsp. ***viscosus***, O.04, h, Switzerland, Valais, Bratsch, 7.7, 46.3*, *M. Bolliger, 0-3* (M), KX958723, KX959220, KX958971; ***Odontites viscosus*** (L.) Clairv., 553, s, Spain, Jaén, Cazorla, near Vadillo Castril, –2.94506, 37.92067, *D. Pinto Carrasco & al., DP 666* (SALA 135716), KX958544, KX959041, KX958792; ***Odontites viscosus***, 554, s, Spain, Palencia, La Pernía, Piedrasluengas, –4.44626, 43.02225, *D. Pinto Carrasco & E. Rico, DP 862* (SALA 137360), KX958545, KX959042, KX958793; ***Odontites viscosus***, 555, s, Spain, León, Cármenes, Genicera, –5.47687, 42.93688, *D. Pinto Carrasco & E. Rico, DP 877* (SALA 137376), KX958546, KX959043, KX958794; ***Odontites vulcanicus*** Bolliger, O.16, h, Morocco, Meknès-Tafilalet, Michlifene, –5.1, 33.4*, *M. Bolliger & D. Moser, 0-M3* (MA 538473), KX958732, KX959229, KX958980; ***Parentucellia latifolia*** (L.) Caruel, 631, s, Spain, Cáceres, Hervás, –5.86475, 40.25480, *M. Martínez Ortega & X. Giráldez Fernández, MO 6019* (SALA 142077), KX958620, KX959117, KX958868; ***Parentucellia latifolia***, 632, s, Spain, Cáceres, Hervás, –5.86475, 40.25480, *M. Martínez Ortega & X. Giráldez Fernández, MO 6019* (SALA 142077), KX958621, KX959118, KX958869; ***Phtheirospermum japonicum*** (Thunb.) Kanitz, P.08, h, Russia, Primorskiy, Okeanskaya (near Vladivostok), 132.0, 43.2*, *A.K. Skvortsov, s.n.* (M), –, KX959248, KX958998; ***Pterygiella tenuisecta*** (Bureau & Franch.) Pinto-Carrasco, E.Rico & M.M.Mart.Ort. [***Phtheirospermum tenuisectum*** Bureau & Franch.], P.07, h, China, Yunnan, Kunming, Heilongtan Botanic Garden, 102.71, 25.08, *B. Dickoré, 13906* (MSB), –, KX959247, –;

(2) Sequences from GenBank

Bartsia alpina L., GB1, JF900503.110, JF900568.110, JF900536.110; ***Bartsia alpina***, GB2, JF900504.110, JF900569.110, JF900537.110; ***Bartsia alpina***, GB3, JF900505.110, JF900570.110, JF900538.110; ***Bartsia alpina***, GB4, JF900502.110, JF900567.110, JF900535.110; ***Bartsia alpina***, GB5, FJ790046.112, FJ790106.112, –; ***Bartsia alpina***, GB6, AM503879.24, AY849600.116, –; ***Bellardia trixago*** (L.) All., GB1, JF900513.110, JF900577.110, JF900545.110; ***Bellardia trixago***, GB2, FJ790063.112, FJ790113.112, KM408301.114; ***Bellardia viscosa*** (L.) Fisch. & C.A.Mey., GB1, JF900514.110, JF900578.110, JF900546.110; ***Bellardia viscosa***, GB2, HM193529.112, HM193525.112, KM408298.114; ***Euphrasia alsa*** F.Muell., GB1, KM408212.114, KC542160.16, KM408282.114; ***Euphrasia nemorosa*** (Pers.) Wallr., GB1, FJ790050.112, FJ790110.112, –; ***Euphrasia officinalis*** L., GB1, JF900506.110, JF900571.110, JF900539.110; ***Euphrasia regelii*** Wettst., GB1, KM408214.114, KC542162.16, KM408285.114; ***Euphrasia rostkoviana*** Hayne, GB1, FJ790052.112, FJ790112.112, –; ***Euphrasia sevanensis*** Juz., GB1, FJ790049.112, FJ790109.112, –; ***Euphrasia stricta*** J.P.Wolff ex J.F.Lehm., GB1, FJ790051.112, FJ790111.112, KM408283.114; ***Hedbergia abyssinica*** (Benth.) Molau, GB1, JF900509.110, JF900574.110, JF900542.110; ***Hedbergia abyssinica***, GB2, KM408231.114, –, KM408293.114; ***Hedbergia abyssinica***, GB3, FJ790061.112, –, KM408291.114; ***Hedbergia abyssinica***, GB4, KM408230.114, –, KM408290.114; ***Hedbergia abyssinica***, GB5, FJ790059.112, FJ790119.112, –; ***Hedbergia decurva*** (Hochst. ex Benth.) A.Fleischm. & Heubl, GB1, JF900511.110, JX629749.110, JF900543.110; ***Hedbergia decurva***, GB2, –, –,

KM408292.114; ***Hedbergia longiflora*** (Hochst. ex Benth.) A.Fleischm. & Heubl subsp. ***longiflora***, GB1, KM408232.114, –, KM408286.114; ***Hedbergia longiflora*** subsp. ***macrophylla*** (Hedberg) A.Fleischm. & Heubl, GB1, JF900519.110, JF900583.110, JF900551.110; ***Hedbergia longiflora***, GB1, JF900510.110, JF900575.110, JX629747.110; ***Lathraea clandestina*** L., GB1, AY911230.115, AF051989.117, –; ***Lathraea clandestina***, GB2, –, JX091325.16, –; ***Lathraea squamaria*** L., GB1, JF900500.110, JF900565.110, JF900533.110; ***Lathraea squamaria***, GB2, FJ790044.112, HM193524.112, KM408309.114; ***Lathraea squamaria***, GB3, AM503877.24, KC542164.16, –; ***Melampyrum arvense*** L., GB1, AM503874.24, JX091327.16, –; ***Melampyrum carstiense*** Fritsch., GB1, GU445314.13, KC542167.16, KM408315.114; ***Melampyrum nemorosum*** L., GB1, FJ797592.112, FJ790117.112, JF900530.110; ***Melampyrum pratense*** L., GB1, FJ790039.112, FJ790099.112, –; ***Melampyrum sylvaticum*** L., GB1, EU624133.112, FJ790104.112, KM408314.114; ***Neobartsia canescens*** (Wedd.) Uribe-Convers & Tank, GB1, JF900518.110, JF900582.110, JF900550.110; ***Neobartsia laniflora*** (Benth.) Uribe-Convers & Tank, GB1, KM408221.114, –, KM408307.114; ***Neobartsia laticrenata*** (Benth.) Uribe-Convers & Tank, GB1, FJ790054.112, FJ790114.112, –; ***Neobartsia mutica*** (Kunth) Uribe-Convers & Tank, GB1, JF900517.110, JF900581.110, JF900549.110; ***Neobartsia pedicularoides*** (Benth.) Uribe-Convers & Tank, GB1, FJ790047.112, FJ790107.112, –; ***Neobartsia ramosa*** (Molau) Uribe-Convers & Tank, GB1, KM408229.114, –, KM408304.114; ***Neobartsia santolinifolia*** (Kunth) Uribe-Convers & Tank, GB1, KM408220.114, –, KM408306.114; ***Neobartsia stricta*** (Kunth) Uribe-Convers & Tank, GB1, KM408222.114, –, KM408305.114; ***Neobartsia tenuis*** (Molau) Uribe-Convers & Tank, GB1, KM408223.114, –, KM408299.114; ***Neobartsia thiantha*** (Diels) Uribe-Convers & Tank, GB1, KM408217.114, –, KM408303.114; ***Nothobartsia asperrima*** (Link) Benedí & Herrero, GB1, JF900508.110, JF900573.110, JF900541.110; ***Nothobartsia asperrima***, GB2, HM193531.112, HM193527.112, –; ***Nothobartsia spicata*** (Ramond) Bolliger & Molau, GB1, JX629746.110, JX629750.110, JX629748.110; ***Odontitella virgata*** (Link) Rothm., GB1, JF900507.110, JF900572.110, JF900540.110; ***Odontites alshehbazianus*** (Dönmez & Mutlu) A.Fleischm. & Heubl, GB1, JF900522.110, JF900586.110, JF900554.110; ***Odontites aucheri*** Boiss., GB1, KM408237.114, –, –; ***Odontites bocconii*** (Guss.) Walp., GB1, HM193532.112, HM193528.112, –; ***Odontites glutinosus*** (M.Bieb.) Benth. [***Macrosyringion glutinosum*** (M.Bieb.) Rothm.], GB1, JF900520.110, JF900584.110, JF900552.110; ***Odontites luteus*** (L.) Clairv., GB1, FJ790045.112, FJ790105.112, –; ***Odontites maroccanus*** Bolliger, GB1, KM408233.114, –, –; ***Odontites pyrenaeus*** (Bubani) Rothm., GB1, JF900527.110, JF900591.110, JF900559.110; ***Odontites rameauanus*** Emb., GB1, JF900523.110, JF900587.110, JF900555.110; ***Odontites vernus*** (Bellardi) Dumort., GB1, KM408236.114, –, KM408287.114; ***Odontites vernus***, GB2, FJ790048.112, FJ790108.112, –; ***Odontites viscosus*** (L.) Clairv., GB1, JF900524.110, JF900588.110, JF900556.110; ***Odontites vulcanicus*** Bolliger, GB1, KM408235.114, –, KM408289.114; ***Parentucellia latifolia*** (L.) Caruel., GB1, JF900515.110, JF900579.110, JF900547.110; ***Parentucellia latifolia***, GB2, HM193530.112, HM193526.112, –; ***Pedicularis groenlandica*** Retz., GB1, HG424130.213, HG423949.213, HQ385148.19; ***Phtheirospermum japonicum*** (Thunb.) Kanitz, GB1, JQ910090.12, JX091332.16, KJ563203.118; ***Phtheirospermum japonicum***, GB2, JQ910089.12, –, –; ***Pseudobartsia glandulosa*** (Bentham) W.B.Yu & D.Z.Li, GB1, JQ910093.12, –, –; ***Pseudobartsia glandulosa***, GB2, GU445317.11, –, –; ***Pterygiella cylindrica*** Tsoong, GB1, JF746385.13, JF746407.13, –; ***Pterygiella cylindrica***, GB2, JF746386.13, JF746408.13, –; ***Pterygiella cylindrica***, GB3, JF746387.13, JF746409.13, –; ***Pterygiella cylindrica*** var. ***suffruticosa*** (D.Y.Hong) L.N.Dong & H.Wang, GB1, JF746399.13, JF746419.13, –; ***Pterygiella cylindrica*** var. ***suffruticosa***, GB2, JF746400.13, JF746420.13, –; ***Pterygiella cylindrica*** var. ***suffruticosa***, GB3, JF746401.13, JF746421.13, –; ***Pterygiella duclouxii*** Franch., GB1, JF746388.13, JF746410.13, KJ563204.118; ***Pterygiella duclouxii***, GB2, JF746395.13, JF746415.13, –; ***Pterygiella duclouxii***, GB3, JF746396.13, JF746416.13, –; ***Pterygiella muliensis*** (C.Y.Wu & D.D.Tao) Pinto-Carrasco, E.Rico & M.M.Mart.Ort. [***Phtheirospermum muliense*** C.Y.Wu & D.D.Tao], GB1, JQ910091.12, –, –; ***Pterygiella nigrescens*** Oliv., GB1, JF746397.12, JF746417.12, –; ***Pterygiella nigrescens***, GB2, JF746398.12, JF746418.12, –; ***Pterygiella nigrescens***, GB3, JF978154.15, KC542177.16, –; ***Pterygiella parishii*** (Hook.f.) Pinto-Carrasco, E.Rico & M.M.Mart.Ort. [***Phtheirospermum parishii*** Hook.f.], GB1, JQ910092.13, –, –; ***Pterygiella tenuisecta*** (Bureau & Franch.) Pinto-Carrasco, E.Rico & M.M.Mart.Ort. [***Phtheirospermum tenuisectum*** Bureau & Franch.], GB1, JF746383.12, –, –; ***Rhinanthus alectorolophus*** (Scop.) Pollich, GB1, JF900501.110, JF900566.110, JF900534.110; ***Rhinanthus crista-galli*** L., GB1, KM408210.114, –, KM408313.114; ***Rhinanthus freynii*** (A.Kern. ex Sterneck) Fiori, GB1, GU445319.12, KC542180.16, KM408310.114; ***Rhinanthus glaciaris*** Person., GB1, FJ790041.112, FJ790101.112, –; ***Rhinanthus kyrollae*** Chabert, GB1, KM408209.114, –, KM408312.114; ***Rhinanthus minor*** L., GB1, FJ790040.112, FJ790100.112, –; ***Rhinanthus rumelicus*** Velen., GB1, FJ790043.112, FJ790103.112, –; ***Rhinanthus serotinus*** (Schönh.) Oborný, GB1, KM408211.114, –, KM408311.114; ***Rhynchocorys elephas*** (L.) Griseb., GB1, FJ790055.112, FJ790115.112, –; ***Rhynchocorys kurdica*** Nábělek, GB1, JF900499.110, JF900564.110, JF900532.110; ***Rhynchocorys maxima*** C.Richter, GB1, FJ790036.112, FJ790096.112, –; ***Rhynchocorys odonthophylla*** Burbidge & Richardson, GB1, FJ790034.112, FJ790094.112, –; ***Rhynchocorys orientalis*** Benth., GB1, JF900498.110, JF900563.110, JF900531.110; ***Rhynchocorys stricta*** (C.Koch) Albov, GB1, FJ790056.112, FJ790116.112, –; ***Seymeria laciniata*** (M.Martens & Galeotti) Standl., GB1, EF103742.111, KC542183.16, EF103820.111; ***Striga asiatica*** (L.) Kuntze, GB1, EU253604.17, AF052000.117, KJ563206.118; ***Tozzia alpina*** subsp. ***carpatica*** Dostál, GB1, FJ790058.112, FJ790118.112, –; ***Tozzia alpina*** L., GB1, JF900512.110, JF900576.110, JF900544.110; ***Tozzia alpina***, GB2, EU259251.18, AF052001.117, KM408280.114; ***Xizangia bartschioides*** (Hand.-Mazz.) 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SUPPLEMENTARY MATERIAL

APPENDIX S1. ITS PSEUDOGENES AND RECOMBINANT SEQUENCES

Materials and methods

Evidence of recombination for the ITS sequences was tested using the pairwise homoplasy index (PHI) test (Bruen & al., 2006) as implemented in SplitsTree v.4 (Huson & Bryant, 2006). Also, the correct order of all different ITS sub-regions (SSU-ITS1-5.8S-ITS2-LSU) was checked with ITSx v.1.0.11 (Bengtsson-Palme & al., 2013). To find pseudogenes, the presence of three conserved 5.8S motifs described for Viridiplantae (Harpke & Peterson, 2008) and the highly conserved ITS1 motif described for flowering plants (Liu & Schardl, 1994) was checked across all newly generated ITS sequences. In addition, the GC contents of ITS1 and ITS2 were calculated separately and compared to detect unbalanced copies (Torres & al., 1990).

Results

The three conserved 5.8S rDNA motifs (Harpke & Peterson, 2008) were present in all newly generated ITS sequences, but two GenBank sequences had single nucleotide mismatches in those areas (*Neobartsia santolinifolia* (Kunth) Uribe-Convers & Tank-KM408220.1 with a substitution in motif 1, and *Striga asiatica*-EU253604.1 with a 1bp indel in motif 2). These slight differences could also have resulted from erroneous sequence editing. The highly conserved ITS1 motif (GGCRY-(4 to 7*n*)-GYGYCAAGGAA; Liu & Schardl, 1994) showed a 5*n* value in all sequences, accounting for a total length of 21 bp. Some substitutions detected are incompatible with the conserved motif. All *Odontites linkii* Heldr. & Sart. ex Boiss. had a T (or Y) in the 3rd position, in all *O. viscosus* (L.) Clairv. sequences an A occupied the 19th position, and in all *Euphrasia* sequences (and one from *Bartsia alpina* L.) the 21st position was a T. Those mutations did not disturb the conserved hairpin structure, and we did not consider them convincing evidence for regarding these sequences as pseudogenes. There was only one sequence (*Rhinanthus rumelicus* Velen.; FJ790043.1) where mutations could possibly prevent hairpin formation. The GC content was stable between individuals in 5.8S, ranging from 52.5 to 56.8%, but varied substantially in ITS1 and ITS2, ranging from 40.4 to 56.3% and from 38.8 to 61.5%,

respectively. To detect sequences that could be regarded as pseudogenes, GC content of ITS1 and ITS2 was compared to those from the same clade. Additionally, we checked whether the GC content of ITS1 and ITS2 in each sequence was balanced (Torres & al., 1990). In this way, only four outlier sequences were assumed to be pseudogenes (*Melampyrum arvense* L., AM503874.2; *M. pratense,* FJ790039.1; *Phtheirospermum parishii* Hook.f., JQ910092.1; *O. sillettii* Brullo, Tomaselli & Wagens., KX958749). However, all seven putatively pseudogenous or erroneously edited ITS samples were placed in the expected clades (i.e., cpDNA clades) and did not distort tree topology. Consequently, they were not excluded from the final alignments. The PHI test did not find statistically significant evidence for recombinant ITS copies (P = 0.8802), and all sequences had all sub-regions in the expected order.

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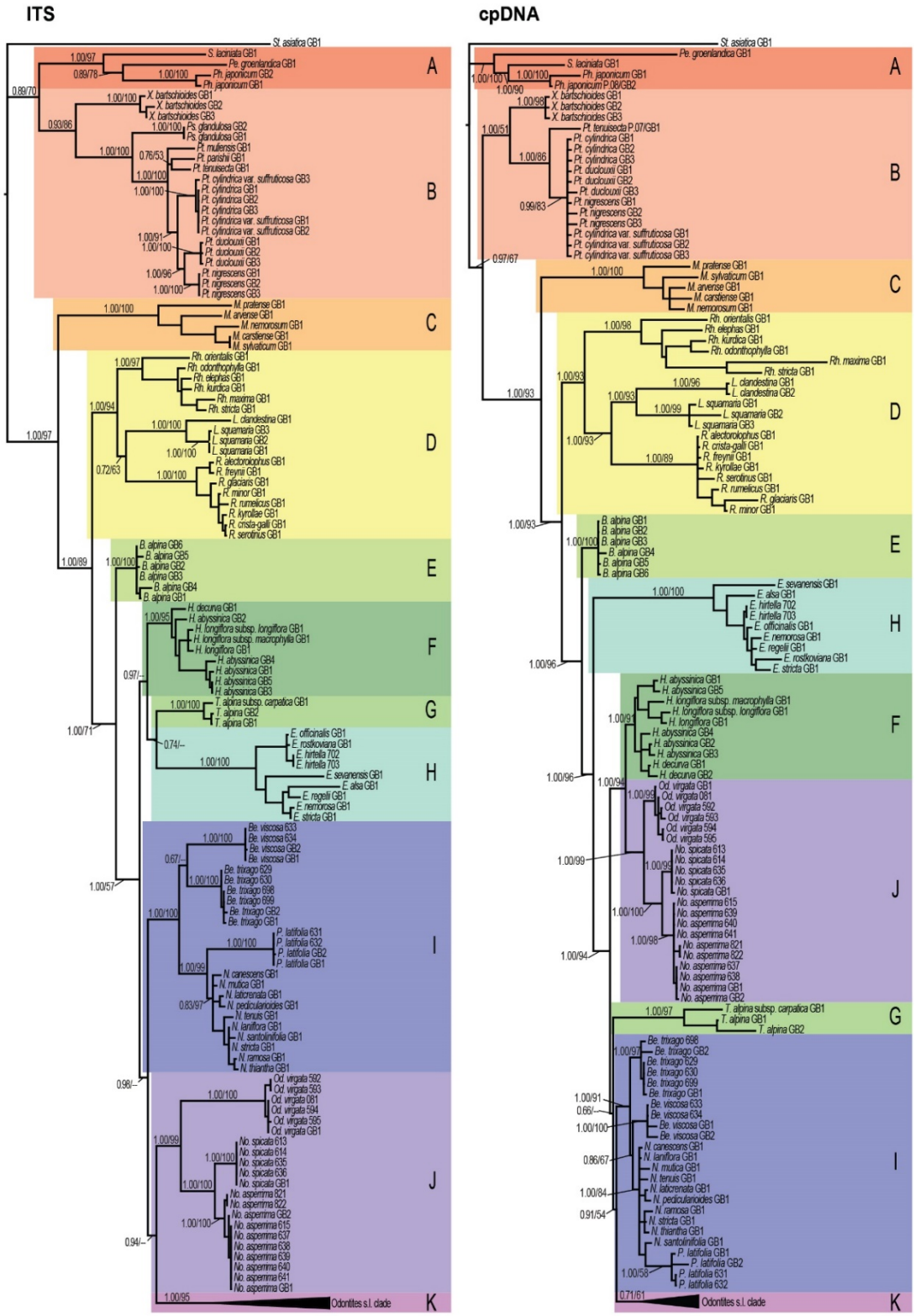
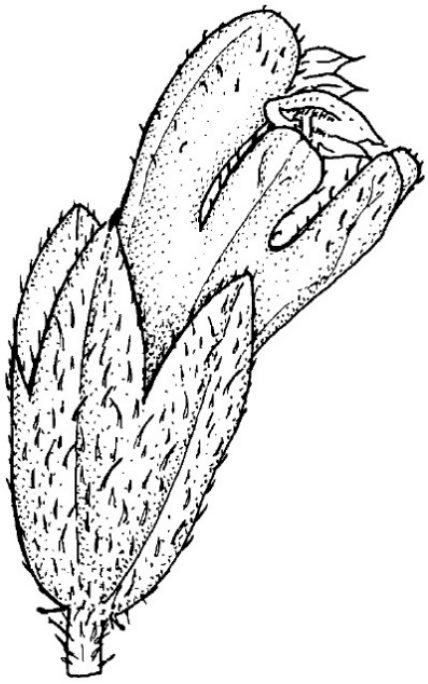


Fig. S1. Majority rule consensus trees from the Bayesian inference analysis of tribe Rhinantheae A) ITS and B) cpDNA datasets, with branch support (PP/BS). Main clades are indicated with different colours and letters. Clade names: A- tribe Pedicularideae, B- Pterygiella Complex II, C- Melampyrum, D- RRL, E- Bartsia s.str., F- Hedbergia, G- Tozzia, H- Euphrasia, I- Bellardia, J- Nothobartsia-Odontitella and K- Odontites. B. = *Bartsia*, Be. = *Bellardia*, E. = *Euphrasia*, H. = *Hedbergia*, L. = *Lathraea*, M. = *Melampyrum*, N. = *Neobartsia*, No. = *Nothobartsia*, Od. = *Odontitella*, P. = *Parentucellia*, Pe. = *Pedicularis*, Ph. = *Phtheirospermum*, Ps. = *Pseudobartsia*, Pt. = *Pterygiella*, R. = *Rhinanthus*, Rh. = *Rhynchocorys*, S. = *Seymeria*, St. = *Striga*, T. = *Tozzia*, X. = *Xizangia*. Synonyms: *Pt. muliensis = Ph. muliense; Pt. parishii = Ph. parishii*; *Pt. tenuisecta = Ph. tenuisectum.*



Capítulo 4: Development of 14 microsatellite markers in *Odontites vernus* s.l. (Orobanchaceae) and cross-amplification in related taxa[1]

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[1] Este capítulo fue publicado en una revista científica antes que el capítulo 2, de modo que aun considerábamos que *Bartsiella* y *Macrosyringion* eran géneros separados de *Odontites.*

ABSTRACT

• *Premise of the study:* Microsatellite primers were developed for the first time in the root hemiparasite herb *Odontites vernus* (Orobanchaceae). These markers will be useful to investigate the role of polyploidization in the evolution of this diploid-tetraploid complex, as well as the extent of gene flow between different ploidy levels.

• *Methods and Results:* Fourteen polymorphic and reproducible loci were identified and optimized from *O. vernus* using a microsatellite-enriched library and 454 Junior sequencing. The set of primers amplified di- to pentanucleotide repeats and showed two to 13 alleles per locus. Transferability was tested in 30 taxa (19 belonging to *Odontites* and 11 from eight other genera of Orobanchaceae tribe Rhinantheae).

• *Conclusions:* The results indicate the utility of the newly developed microsatellites in *O. vernus* and several other species, which will be useful for taxon delimitation and conservation genetics studies.

Keywords: conservation; diploid-tetraploid complex; microsatellite; *Odontites vernus*; Orobanchaceae; Rhinantheae.

RESUMEN

• Premisa del estudio: Se desarrollaron por primera vez cebadores de marcadores microsatélite en la planta herbácea hemiparásita *Odontites vernus* (Orobanchaceae). Estos marcadores serán útiles para investigar el papel de la poliploidización en la evolución de este complejo diploide-tetraploide, así como la extensión del flujo genético entre diferentes niveles de ploidía.

• Métodos y resultados: Se identificaron y optimizaron catorce loci polimórficos y reproducibles en *O. vernus,* utilizando una librería enriquecida en regiones microsatélite y secuenciación de nueva generación (454 Junior). El conjunto de cebadores amplificó regiones cuyo motivo repetitivo es de entre 2 y 5 nucleótidos, y se obtuvieron de 2 a 13 alelos por locus. La transferibilidad se probó en 30 taxones (19 pertenecientes a *Odontites* y 11 de otros 8 géneros de la tribu Rhinantheae, Orobanchaceae).

• Conclusiones: Los resultados indican la utilidad de los microsatélites recientemente desarrollados en *O. vernus* y en varias otras especies, y serán útiles en estudios delimitación de taxones y de conservación genética de especies amenazadas.

Palabras clave: complejo diploide-tetraploide; conservación; microsatélite; *Odontites vernus*; Orobanchaceae; Rhinantheae.

INTRODUCTION

The predominantly Mediterranean genus *Odontites* Ludw. (Orobanchaceae; Bennett and Mathews, 2006) comprises ca. 26 species of annual and perennial root hemiparasites (Bolliger, 1996) growing in grasslands, shrublands, and wood edges. It includes weeds (Parker, 2013), as well as species listed on national and regional catalogs of endangered plants (e.g., López Udías and Fábregat Llueca, 2010), registered on the International Union for the Conservation of Nature Red List (http://www.iucnredlist.org/), or with narrow distribution areas (Bolliger, 1996).

The *O. vernus* (Bellardi) Dumort. group, which includes three species, is the most widespread of the genus, occupying the temperate regions of Eurasia with one population in northern Morocco (Bolliger, 1996). However, phylogenetic relationships and evolutionary patterns within the group remain largely unclear due to a complex interplay between the diploid-tetraploid cytotypic variation and seasonal ecotypes differing in morphology (Koutecký et al., 2012). *Odontites vernus* sensu lato (s.l.; Rico, 2009) includes diploid and tetraploid individuals. The latter are probably of autopolyploid origin, as no distinct subgenomes were found in the karyotype (Delgado et al., 2015) and morphology is not intermediate between any two known diploid species. However, the hypothesis of an autopolyploid origin has not been addressed using genetic markers. Furthermore, it is not clear whether some levels of gene flow are maintained in locations where diploids and tetraploids co-occur (Snogerup, 1983; Koutecký et al., 2012). Although it is known that *O. vernus* can self-pollinate (Nilsson and Alves-dos-Santos, 2009), inbreeding rates in populations remain unknown. Therefore, genetic markers are needed to study gene flow patterns and how populations of *O. vernus* are connected. Furthermore, the transferability of the loci to other species of the genus would bring new information for taxonomic revision of *Odontites* species and conservation of endemic and/or threatened taxa.

METHODS AND RESULTS

***Microsatellite development*—**Silica gel–dried leaves of two diploid individuals of *O. vernus* (see Appendix 1 for voucher information) were selected for genomic DNA extraction using Invisorb Spin Plant Mini Kit (Invitek, Berlin, Germany). Ploidy level was checked with a CyFlow flow cytometer (Partec GmbH, Münster, Germany), using 'Woody Plant Buffer' (WPB; Loureiro et al., 2007) and *Solanum pseudocapsicum* L. as the internal standard (Temsch et al., 2010). DNA extraction was enriched with AC, AG, TGT, and CCT motifs following Nunome et al. (2006). The resulting microsatellite library was sequenced using a 454 GS Junior Sequencer (454 Life Sciences, a Roche Company, Branford, Connecticut, USA). Analyses with

QDD software (Meglécz et al., 2010) revealed 4335 sequence reads with microsatellite motifs (from a total of 16,050), and primer pairs were designed for 169 regions. A set of 36 primer pairs with low penalty, different lengths, and containing different repeat motifs was tested. Amplification was evaluated in four diploid and three tetraploid individuals of *O. vernus*. PCRs were performed in 12.5-μL reactions, which contained 45.5 ng of DNA, 1× PCR buffer (Biotools, Madrid, Spain), 1.5 mM $MgCl_2$ (Biotools), 0.2 mM of each dNTP (Life Technologies, Carlsbad, California, USA), 0.33 mM of each primer (Eurofins, Ebersberg, Germany), and 0.5 unit of DNA Polymerase (Biotools), using the following conditions: an initial step at 94°C for 2 min; followed by 35 cycles of 1 min at 94°C, 1 min at primer-specific annealing temperature, and 50 s at 72°C; and a final extension of 15 min at 72°C. PCR products were visualized on a 2.5% agarose gel.

PCR products were sequenced by Macrogen Europe (Amsterdam, The Netherlands), and the obtained sequences were checked for homology to the expected region. Consistent amplification and levels of polymorphisms were analyzed in gel images. Eighteen loci were selected (see Appendix 2 for discarding reasons) and tested on 140 *O. vernus* samples using a three-primer PCR protocol (Schuelke, 2000) with the universal primer M13(–21) 5′-TGTAAAACGACGGCCAGT-3′ marked with 5-FAM, VIC, NED, or PET fluorescent dyes (Life Technologies; Table 1). The PCR mix was as described above, except that 0.2 mM of each reverse and fluorescent-labeled M13 primer and 0.08 mM of the forward primer were used. Cycling conditions were also as described above, adding 10 cycles of 1 min at 94°C, 1 min at 53°C, and 50 s at 72°C before the final extension. Pooled PCR products were run on an ABI 3730 Capillary Sequencer (Life Technologies) using GeneScan 500 LIZ Size Standard (Life Technologies). Electropherograms were analyzed with GeneMarker AFLP/Genotyping Software version 1.8 (SoftGenetics, State College, Pennsylvania, USA). Three loci were discarded due to genotyping difficulties, and an additional one was monomorphic. Because lengths of some alleles differed from expected sizes, alleles found in homozygous individuals were sequenced to verify indel presence and/or imperfect microsatellite motifs. Indel presence was confirmed in all but three loci (Ov-19, Ov-21, and Ov-35), and imperfect microsatellite motifs were confirmed in two loci (Ov-5 and Ov-25). Additionally, denaturation temperature (T_d) was reduced to 83°C to test if lower T_d improved genotyping (Olejniczak and Krzyzosiak, 2006). Of the remaining 14 loci, T_d = 83 produced better results for two loci, in two cases there were no differences, and in 10 loci there was reduced scorability, contrary to expectations.

TABLE 1. Characteristics of 14 polymorphic microsatellite loci developed in *Odontites vernus*.

Locus	Primer sequences (5′-3′)	Fluorescent label	Repeat motif	Allele size range (bp)[a]	*A*	Indel detected	T_a (°C)	T_d (°C)	GenBank accession no.[b]
Ov-2	F: CCCAAGTTTGTTAATTGGATCG R: GAACTGCAGCTGGAACCTCTA	VIC	$(AATT)_9$	171–213	11	Y	54	94	KT777566–KT777574
Ov-5	F: ATTAGGTACAACCACACGAGGG R: ATACTCGGCATCTTGCAATTCT	VIC	$(TA)_4$-$(CA)_8$	178–190	6	N	55	94	KT777577–KT777579
Ov-6	F: CACTCTCCCACGTTTCTTGATT R: TCAGAAATGGGGTATGAGAAAA	NED	$(AGC)_6$	92–116	13	Y	54	94	KT777580–KT777583
Ov-10	F: TGAATAATGTTTTCAGTCCATAC R: CACACTCTTGTAGCTATGTGCC	PET	$(AGT)_6$	213–217	4	Y	55	94	KT777587–KT777590
Ov-15	F: CTAGGGTTTGGGAATGTGGTT R: CCTAGCTACCCAGATACCATCC	NED	$(ACT)_5$	93–108	11	Y	54	*	KT777593–KT777596
Ov-17	F: TATCGATCCACTCGTGAAACAC R: TTCAGATCACGGTACACGATTC	PET	$(AAAAC)_5$	309–327	4	Y	55	94	KT777597–KT777599
Ov-19	F: GAGGAGGATTGAGGATTGCATA R: CCCACCATTTCATTTACTCTCC	5-FAM	$(AGGG)_5$	85–94	7	—	55	94	KT777600
Ov-20	F: GAGGAGACCCAATAACAAAATTA R: AATTTAAGCACCATGTTGAA	PET	$(AGTT)_3$	98–122	5	Y	55	83	KT777601–KT777603
Ov-21	F: GATCCATTAGCAATGGGACTTT R: TCCAGGTCAAACAGTGAACAAC	NED	$(AG)_{11}$	238–274	13	N	55	94	KT777604–KT777613
Ov-25	F: CTGCCATAGATAACATGCCAAA R: CCCCATGCCGAGAAAG	5-FAM	$(TA)_3$-$(CA)_9$	185–196	8	Y	55	94	KT777617–KT777620
Ov-28	F: ACAAGATTCTTCCCTCCCTGTC R: ATCCATGTGAGCAATGATGAAA	5-FAM	$(AC)_6$	227–258	8	Y	55	*	KT777622–KT777626
Ov-30	F: TTCGGATATCGAATAAAATGGG R: TCGCAATGTTTCCTGTTGTTAC	NED	$(AC)_5$	249–257	6	Y	55	94	KT777627–KT777629
Ov-33	F: CCTTGAACATACAGAAACTACAACAA R: TGATTACTATGCAACCACTGCC	VIC	$(AC)_5$	367–387	3	Y	55	94	KT777631–KT777633
Ov-35	F: TCAAATTCATTAGAACTGCGTCA R: CTATTTGACCATGAGCTCCACC	PET	$(ACC)_5$	310–313	2	N	55	83	KT777634–KT777635

Note: *A* = number of alleles; T_a = optimal annealing temperature; T_d = optimal denaturation temperature.
[a] Range of fragment sizes included the M13(–21) tail attached to the forward primer. Sizes were taken from GeneMarker allele calls.
[b] More than one accession per locus (except for Ov-19) in order to check imperfect microsatellite motifs and/or indel presence.
* No differences in genotyping between both T_d tested.

***Population genetic parameters in two populations of* Odontites vernus—** Two populations were selected to obtain population genetic parameters that could be illustrative of performance in two different situations. In one population (Tejada), all sampled individuals were diploids, but in the other one (San Miguel del Arroyo [SMA]) 32 were diploids and 36 were tetraploids. The number of alleles per locus, observed and expected heterozygosity, significance of deviation from Hardy–Weinberg equilibrium (HWE; Table 2), and test for linkage disequilibrium between markers were estimated using Arlequin version 3.5.1.2 (Excoffier and Lischer, 2010). To perform those analyses, allele sizes were not transformed into number of repeats, and exact allele dosage was not estimated in tetraploids. In SMA, these parameters were calculated only for diploids. The number of alleles per locus ranged from two to 13 in the complete data set (Table 1), but varied from one to five in the two selected populations (Table 2). Four loci were monomorphic in both populations, and four to six were polymorphic in the studied populations. Significant deviation from HWE ($P < 0.05$) was found in all loci probably due to inbreeding, as recorded in the closely related genus *Euphrasia* L. (French et al., 2003). Linkage disequilibrium was significant after Bonferroni correction in all pairwise comparisons, except those involving allele Ov-19 and the pair Ov-10/Ov-15. Regarding alleles related to ploidy levels, almost all alleles in every locus are shared between ploidy levels overall. But in the SMA samples, there are six loci (Ov-5, Ov-19, Ov-21, Ov-28, Ov-30, Ov-33) that differentiate ploidies unequivocally.

TABLE 2. Results of initial screening of within-population variation in two populations of *Odontites vernus*.

	Tejada ($n = 30$)				SMA diploids ($n = 32$)				SMA tetraploids ($n = 36$)		
Locus	A	H_o	H_e	HWE[a]	A	H_o	H_e	HWE[a]	A	$A_{per\ ind.}$	H_o[b]
Ov-2	2	0.16667	0.34520	0.01190*	3	0.06250	0.63641	0.00000***	1	—	—
Ov-5	1	—	—	—	1	—	—	—	2	2	1.00
Ov-6	1	—	—	—	1	—	—	—	3	3	1.00
Ov-10	1	—	—	—	2	0.15625	0.48363	0.00013***	1	—	—
Ov-15	4	0.10000	0.29774	0.00000***	2	0.18750	0.49008	0.00080***	3	3	1.00
Ov-19	2	0.00000	0.12655	0.00090***	1	—	—	—	2	2	1.00
Ov-20	1	—	—	—	2	0.18750	0.50000	0.00068***	1	—	—
Ov-21	2	0.10000	0.46271	0.00003***	5	0.21875	0.65278	0.00000***	1	—	—
Ov-28	1	—	—	—	1	—	—	—	3	3	1.00
Ov-33	1	—	—	—	1	—	—	—	2	2	1.00

Note: — = monomorphic loci; A = number of alleles; $A_{per\ ind.}$ = maximum number of alleles in a single individual; H_e = expected heterozygosity; H_o = observed heterozygosity; HWE = Hardy–Weinberg equilibrium probabilities; n = number of individuals sampled.

[a] Deviations from HWE were statistically significant at $*P < 0.05$ and $***P < 0.001$. Note that there were no deviations at $P < 0.01$.

[b] As it is not possible to calculate H_o accurately for tetraploids, the proportion of individuals with more than one allele is shown.

***Cross-amplification in other* Odontites *species and related genera*—**The 18 selected loci were tested in 19 *Odontites* taxa and 11 other taxa from eight related genera using the PCR conditions described above. Fragment separation results (Table 3) were promising in closely related species (*O. corsicus* (Loisel.) G. Don, *O. hollianus* (Lowe) Benth., *O. luteus* (L.) Clairv., *O. kaliformis* (Pourr. ex Willd.) Pau, and *O. recordonii* Burnat & Barbey) because they amplify in 13 to 17 loci, and sometimes showed more than one allele, despite a small sample size (n =

4). Furthermore, good results were obtained for several other taxa– locus combinations. Development of species-specific PCR protocols could improve these results, especially in some other *Odontites* species (i.e., *O. bolligeri* E. Rico, L. Delgado & Herrero, *O. pyrenaeus* (Bubani) Rothm., and *O. cebennensis* H. J. Coste & Soulié).

CONCLUSIONS

A set of polymorphic microsatellite markers for *O. vernus* is reported for the first time. Successful results for these loci in the cross-amplification tests extend their potential usefulness to other closely related taxa. These markers will be useful for investigating genetic diversity in threatened species, self-pollination rates, origin and evolution of polyploidy, and ecotypic variation and local adaptation in populations.

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TABLE 3. Results of cross-amplification within the genus *Odontites* and related genera.[a]

Species[b]	*n*	Ov-2	Ov-3[c]	Ov-5	Ov-6	Ov-10	Ov-12[c]	Ov-15	Ov-17	Ov-19	Ov-20	Ov-21	Ov-25	Ov-26[c]	Ov-28	Ov-30	Ov-32[c]	Ov-33	Ov-35
B. inaequalis Benth.	1									0/0/1 (1)		0/0/1 (1)	0/0/1 (1)			0/0/1 (1)			
Ba. rameauana (Emb.) Bolliger	4		2/1/1 (2)	3/0/1 (1)			1/0/3 (4)			0/0/4 (2)			0/0/4 (1)		0/0/4 (3)			1/3/0 (2)*	
Be. trixago (L.) All.	2									0/0/2 (1)		0/0/2 (1)	0/0/2 (2)						
Eu. antarctica Benth.	1									0/0/1 (1)			0/1/0 (1)						
Eu. hirtella Jord. ex Reut.	1		0/0/1 (1)							0/0/1 (1)		0/0/1 (1)	0/1/0 (1)						
Ma. longiflorum (Lam.) Rothm.	4	3/0/1 (1)	3/0/1 (1)	0/0/4 (1)			3/0/1 (2)			0/2/2 (1)			0/0/4 (2)		0/1/3 (1)*	3/0/1 (1)		3/0/1 (1)*	
No. asperrima (Link) Benedí & Herrero	4				0/0/4 (1)		1/0/3 (1)			0/0/4 (1)			0/0/4 (1)						2/2/0 (2)
No. spicata (Ramond) Bolliger & Molau	4		3/0/1 (1)	3/1/0 (1)			0/0/4 (1)			0/0/4 (1)			0/0/4 (1)						1/2/1 (1)
Od. virgata (Link) Rothm.	4		2/0/2 (2)				3/0/1 (1)			0/0/4 (1)			0/0/4 (2)		3/0/1 (1)				
O. bocconii (Guss.) Walp.	4	2/0/2 (1)	3/0/1 (1)			0/0/4 (3)*				0/0/4 (2)			0/0/4 (1)	2/2/0 (1)	0/0/4 (3)			1/2/1 (1)*	
O. bolligeri E. Rico, L. Delgado & Herrero	4	3/0/1 (1)	2/0/2 (1)			1/0/3 (1)	0/0/4 (1)			0/0/4 (2)			0/0/4 (1)		0/2/2 (4)	2/2/0 (1)	3/1/0 (1)	0/0/4 (1)*	
O. cebennensis H. J. Coste & Soulié	4		3/0/1 (1)		3/1/0 (1)	0/0/4 (1)	0/0/4 (3)		1/1/2 (1)	0/0/4 (2)			0/0/4 (1)		0/0/4 (2)	0/3/1 (3)		0/0/4 (1)	
O. corsicus (Loisel.) G. Don	4	0/0/4 (1)	0/0/4 (3)	0/0/4 (1)	0/0/4 (1)		0/0/4 (2)		0/1/3 (2)	0/0/4 (1)		3/0/1 (1)	0/0/4 (1)	0/0/4 (1)	0/0/4 (2)	0/0/4 (2)	1/3/0 (1)	1/0/3 (1)	0/0/4 (1)
O. foliosus Pérez Lara	4					0/0/4 (2)				0/0/4 (2)			0/0/4 (1)		1/0/3 (1)			2/1/1 (3)*	
O. hollianus (Lowe) Benth.	4	0/0/4 (2)	0/0/4 (1)	0/0/4 (2)	0/0/4 (1)	1/0/3 (1)				0/0/4 (1)		0/0/4 (1)	0/0/4 (1)	0/1/3 (1)	0/0/4 (2)	1/0/3 (1)		0/0/4 (1)	
O. kaliformis (Pourr. ex Willd.) Pau	4	0/0/4 (1)	0/0/4 (1)	3/1/0 (1)	0/0/4 (1)	0/0/4 (1)	0/0/4 (1)	0/0/4 (3)	1/2/1 (1)	0/0/4 (1)		0/0/4 (1)	0/0/4 (1)	0/0/4 (1)	0/1/3 (1)	0/0/4 (1)		0/1/3 (1)	0/0/4 (1)
O. linkii Heldr. & Sartori ex Boiss.	4					0/1/3 (1)			3/1/0 (1)	0/0/4 (1)			0/1/3 (1)		0/0/4 (2)	3/1/0 (1)		2/2/0 (1)*	
O. luteus (L.) Clairv.	4	2/2/0 (1)	0/0/4 (5)	0/0/4 (2)	0/0/4 (2)	0/0/4 (2)	0/0/4 (5)		0/1/3 (2)	0/0/4 (4)		2/0/2 (4)	0/0/4 (2)	0/0/4 (2)	0/0/4 (4)	0/0/4 (2)		0/0/4 (1)	0/1/3 (5)
O. maroccanus Bolliger	4	3/0/1 (1)							1/1/2 (2)	0/0/4 (1)			0/1/3 (1)		1/0/3 (1)	2/1/1 (1)		2/2/0 (2)*	
O. powellii Maire	4								3/1/0 (1)	0/0/4 (2)			0/0/4 (2)		0/2/2 (2)			1/3/0 (3)*	
O. pyrenaeus subsp. *abilianus* P. Monts.	4		2/0/2 (1)	2/2/0 (1)		0/0/4 (1)	0/0/4 (2)	3/0/1 (2)		0/0/4 (2)			0/0/4 (1)		0/0/4 (1)	2/0/2 (1)		0/0/4 (2)	
O. pyrenaeus (Bubani) Rothm. subsp. *pyrenaeus*	4	3/0/1 (1)	2/0/2 (3)	1/1/2 (2)		0/0/4 (2)	0/1/3 (6)	1/0/3 (2)	2/2/0 (1)	0/0/4 (1)			0/0/4 (2)		0/0/4 (2)	3/1/0 (1)		0/0/4 (2)	
O. recordonii Burnat & Barbey	4	0/0/4 (3)	0/0/4 (4)	0/0/4 (4)	0/0/4 (2)	0/1/3 (2)	0/0/4 (4)	0/0/4 (1)	0/0/4 (2)	0/0/4 (3)		0/0/4 (4)	0/0/4 (1)	0/0/4 (1)	0/0/4 (2)	0/0/4 (1)		1/0/3 (1)	0/0/4 (1)

TABLE 3. Continued.

Species[b]	*n*	Ov-2	Ov-3[c]	Ov-5	Ov-6	Ov-10	Ov-12[c]	Ov-15	Ov-17	Ov-19	Ov-20	Ov-21	Ov-25	Ov-26[c]	Ov-28	Ov-30	Ov-32[c]	Ov-33	Ov-35
O. viscosus subsp. *asturicus* M. Laínz	2			1/1/0 (1)					1/0/1 (1)	0/0/2 (1)			0/0/2 (1)		1/1/0 (1)	0/2/0 (1)			
O. viscosus subsp. *australis* (Boiss.) Jahand. & Maire	2		1/0/1 (1)				1/0/1 (1)		0/2/0 (1)	0/0/2 (2)			0/0/2 (2)			1/0/1 (1)			
O. viscosus subsp. *granatensis* (Boiss.) Bolliger	2		1/1/0 (1)	1/0/1 (1)					0/0/2 (1)	1/0/1 (1)			0/0/2 (2)			1/1/0 (1)			
O. viscosus subsp. *lusitanicus* Bolliger	2		1/0/1 (1)	0/2/0 (1)			0/1/1 (2)		0/0/2 (1)	0/0/2 (1)			0/0/2 (1)						
O. viscosus (L.) Clairv. subsp. *viscosus*	2		1/0/1 (1)	0/1/1 (2)			0/0/2 (1)		0/0/2 (1)	0/0/2 (1)			0/0/2 (1)			1/0/1 (1)			
Pa. latifolia (L.) Caruel	2		1/0/1 (1)		1/0/1 (1)					0/0/2 (2)			0/0/2 (1)						
Pa. viscosa (L.) Caruel	2		1/0/1 (2)			1/1/0 (1)*	0/2/0 (1)		0/1/1 (1)	0/0/2 (1)		0/0/2 (2)	0/0/2 (2)					1/0/1 (1)*	

Note: *n* = number of individuals sampled.

[a]Amplification success is presented as: number of individuals that did not amplify/number of individuals that amplified weakly/number of individuals that amplified successfully (number of alleles detected). No amplification = peak height >0 and <250 relative fluorescence units (RFU); weak amplification = peak height >250 and <1000 RFU; successful amplification = peak height >1000 RFU; * = presence of spurious peak. Empty cells indicate failed amplification in all individuals.

[b]Abbreviations: *B.* = *Bartsia*; *Ba.* = *Bartsiella*; *Be.* = *Bellardia*; *Eu.* = *Euphrasia*; *Ma.* = *Macrosyringion*; *No.* = *Nothobartsia*; *Od.* = *Odontitella*; *O.* = *Odontites*; *Pa.* = *Parentucellia.*

[c]Loci excluded in *Odontites vernus* due to genotyping difficulties or no polymorphism.

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APPENDIX 1

Appendix 1. Voucher information for *Odontites* and related genera samples used in this study.

Species	Collector no. and voucher accession[a,b]	*n*[c]	Collection locality	Coordinates[d]
Bartsia inaequalis Benth.	S. Pfanzelt 999, SALA 153256	1	Bolivia: La Paz, Takesi valley	19KFB2480
Bartsiella rameauana (Emb.) Bolliger	AQ 2129, MA 746138	2	Morocco: Azilal, Jbel Tarkeddit	29RQQ3692
Bartsiella rameauana	VL 172, SALA 149231	2	Morocco: Ouarzazate, Tizi n'Ait Hamad	29RQQ5992
Bellardia trixago (L.) All.	DP 918, SALA 142076	1	Spain: Burgos, Castrillo de la Vega	30TVM3411
Bellardia trixago	MO 6020, SALA 142078	1	Spain: Cáceres, Gabriel y Galán Reservoir	29TQE4456
Euphrasia antarctica Benth.	S. Pfanzelt 699, CONC 180033	1	Chile: Magallanes, San Juán	19FCA7056
Euphrasia hirtella Jord. ex Reut.	ER 8041, SALA 142118	1	Spain: Ávila, San Martín de la Vega del Alberche	30TUK1778
Macrosyringion longiflorum (Lam.) Rothm.	DP 11, SALA 135639	1	Spain: Burgos, Castrillo de la Vega	30TVM3508
Macrosyringion longiflorum	DP 851, SALA 137313	1	Spain: Soria, Aldehuela de Periañez	30TWM5429
Macrosyringion longiflorum	DP 898, SALA 137290	1	Spain: Segovia, Ayllón	30TVL8073
Macrosyringion longiflorum	VL 82, SALA 137638	1	Morocco: Chefchaouen, Jbel L'akraa	30SUD0490
Nothobartsia asperrima (Link) Benedí & Herrero	DP 1062, SALA 156176	1	Morocco: Chefchaouen, track betw. Sidi Jel and Beni Bouker	30SUD0696
Nothobartsia asperrima	ER 7909, SALA 123313	1	Portugal: Ribatejo, Tomar	29SND4983
Nothobartsia asperrima	MS 958, SALA 123310	1	Portugal: Estremadura, Azeitão	29SNC0164
Nothobartsia asperrima	MS 960, SALA 123311	1	Portugal: Estremadura, Sesimbra	29SMC8151
Nothobartsia spicata (Ramond) Bolliger & Molau	ER 7920, SALA 125802	2	Spain: Oviedo, Ribadesella	30TUP3611
Nothobartsia spicata	ER 7921, SALA 125801	2	Spain: Santander, Peñarrubia	30TUN6791
Odontitella virgata (Link) Rothm.	DP 14, SALA 135636	1	Spain: Burgos, Castrillo de la Vega	30TVM3610
Odontitella virgata	ER 7959, SALA 136278	1	Spain: Cádiz, Los Barrios	30STF6712
Odontitella virgata	LD 1069, SALA 136280	1	Spain: A Coruña, Santiso	29TNH8046
Odontitella virgata	SA 297, SALA 135467	1	Portugal: Beira Litoral, rd. betw. Mira and Castanhede	29TNE2771
Odontites bocconii (Guss.) Walp.	G. Domina s.n., PAL 90581	2	Italy: Sicilia, San Martino delle Scale	33SUC4716
Odontites bocconii	JPG-11-03, SALA 142125	2	Italy: Sicilia, Madonie Regional Natural Park	33SVB1389
Odontites bolligeri E. Rico, L. Delgado & Herrero	AQ 2812, SALA 142142	1	Morocco: Berkane, Béni-Snassen	30SWD5652
Odontites bolligeri	DP 832, SALA 136804	1	Spain: Málaga, Frigiliana	30SVF1970
Odontites bolligeri	MO 4566, SALA 135619	1	Spain: Granada, Restábal	30SVF4886
Odontites bolligeri	VL 153, SALA 156172	1	Spain: Almería, Láujar de Andarax	30SWF1094
Odontites cebennensis H. J. Coste & Soulié	DP 628, SALA 135679	1	Spain: Barcelona, La Pobla de Lillet	31TDG1877
Odontites cebennensis	DP 1760, SALA 156184	1	Andorra: Ordino, track to Castell dels Moros	31TCH8012
Odontites cebennensis	DP 1842, SALA 156185	1	Spain: Gerona, Albanyà	31TDG7578
Odontites cebennensis	DP 1894, SALA 156186	1	Spain: Teruel, Linares de Mora	30TYK0665
Odontites corsicus (Loisel.) G. Don	A. Tribsch s.n., SALA 137639	4	France: Corse, Bastia	32TNN3133
Odontites foliosus Pérez Lara	DP 821, SALA 156297	1	Spain: Málaga, Manilva	30STF9724
Odontites foliosus	ER 7903, SALA 103775[e]	1	Spain: Cádiz, Barbate	30STF3408
Odontites foliosus	ER 7939, SALA 134536	1	Spain: Cadiz, Puerto Real	29SQA5645
Odontites foliosus	VL 135, SALA 144130	1	Spain: Málaga, Genalguacil	30STF9947
Odontites hollianus (Lowe) Benth.	SC 17379, MA 714540	1	Portugal: Madeira, betw. Pico do Arieiro and Pico Ruivo	28SCB1823
Odontites hollianus	M. Díaz s.n., SALA 156496	2	Spain: Santa Cruz de Tenerife, Isla de La Palma	28RBS1482
Odontites hollianus	MS 5056, SALA 125030	1	Portugal: Madeira, betw. O Ninho da Manta and O Pico Cidrão	28SCB1724
Odontites kaliformis (Pourr. ex Willd.) Pau	ER 7913, SALA 124706	2	Spain: Valencia, Sagunto	30SYJ3690
Odontites kaliformis	ER 7914, SALA 124707	2	Spain: Castellón, Cabanes	31TBE6052
Odontites linkii Heldr. & Sartori ex Boiss.	AH 3359, SALA 140386	1	Greece: Peloponnese, Ahaia	34SFH0215
Odontites linkii	AH 3480, SALA 140486	1	Greece: Peloponnese, Korinthia	34SFH2804
Odontites linkii	CA 14257, SALA 140800	2	Greece: Peloponnese, Lakonia	34SFG1806
Odontites luteus (L.) Clairv.	BR 187, SALA 142123	1	Czech Republic: Jihomoravsk kraj, betw. Klentnice and Mikulov	33UXQ2010
Odontites luteus	DP 763, SALA 137330	1	Spain: Albacete, Riópar	30SWH5361
Odontites luteus	DP 1018, SALA 110042	1	Spain: Valladolid, Santibañez de Valcorba	30TUM7904
Odontites luteus	ER 7852, SALA 136275	1	Spain: Lérida, betw. Puente de Montañana and Tremp	31TCG1670
Odontites maroccanus Bolliger	DP 785, SALA 156299	1	Morocco: Ifrane, Tizi-n-Tretten	30SUC1003
Odontites maroccanus	DP 1082, SALA 156177	1	Morocco: Ifrane, Aïn Vittel	30SUC0314
Odontites maroccanus	DP 1084, SALA 156178	1	Morocco: Ifrane, Michlifen	30SUB0699

Appendix 1. Continued.

Species	Collector no. and voucher accession[a,b]	n[c]	Collection locality	Coordinates[d]
Odontites maroccanus	NLG 56, SALA 156170	1	Morocco: Ifrane, near Michlifen	30SUB0498
Odontites powellii Maire	AQ 2119, MA 746128	1	Morocco: Béni-Mellal, Tizzi-n-Aif	29SQS8002
Odontites powellii	DP 786, SALA 156298	1	Morocco: Ifrane, Tizi-n-Tretten	30SUC1003
Odontites powellii	NLG 64, SALA 156171	1	Morocco: Khénifra, Col du Zad	30SUB0750
Odontites powellii	VL 83, SALA 156300	1	Morocco: Chefchaouen, Jbel L'akraa	30SUD0490
Odontites pyrenaeus subsp. *abilianus* P. Monts.	DP 1603, SALA 156179	1	Spain: Huesca, Jaca	30TXN9312
Odontites pyrenaeus subsp. *abilianus*	DP 1607, SALA 156180	1	Spain: Zaragoza, Longás	30TXN6905
Odontites pyrenaeus subsp. *abilianus*	DP 1615, SALA 156181	1	Spain: Huesca, Jaca	30TYN0614
Odontites pyrenaeus subsp. *abilianus*	ER 7746, SALA 103068	1	Spain: Huesca, Jaca	30TXN9707
Odontites pyrenaeus (Bubani) Rothm. subsp. *pyrenaeus*	DP 615, SALA 135664	1	Spain: Lérida, Sarroca de Bellera	31TCG2492
Odontites pyrenaeus subsp. *pyrenaeus*	DP 1667, SALA 156182	1	Spain: Huesca, Isábena	31TCG0387
Odontites pyrenaeus subsp. *pyrenaeus*	DP 1736, SALA 156183	1	Spain: Lérida, Cabó	31TCG5375
Odontites pyrenaeus subsp. *pyrenaeus*	ER 7845, SALA 136276	1	Spain: Huesca, Plan	31TBH7515
Odontites recordonii Burnat & Barbey	DP 607, SALA 135656	1	Spain: Vitoria, Elciego	30TWN3008
Odontites recordonii	DP 672, SALA 135722	1	Spain: Albacete, Socovos	30SWH9242
Odontites recordonii	DP 692, SALA 135742	1	Spain: Guadalajara, Fuentelviejo	30TWK0184
Odontites recordonii	LD 1019, SALA 135629	1	Spain: Lérida, Sanaüja	31TCG6136
Odontites vernus (Bellardi) Dumort.	A. Tribsch 4650, SALA 126029	1	Austria: Land Salzburg, Salzburg	33TUN5199
Odontites vernus	BR 27, SALA 135614	2	Bulgaria: Veliko Tarnovo, betw. Dobre Dyal and Rodina	35TMH0972
Odontites vernus	BR 127, SALA 137352	1	Serbia: Moravica, Čačak	34TDP3960
Odontites vernus	BR 158, SALA 142120	2	France: Haute-Normandie, near St. Sebastien	31UCQ6131
Odontites vernus	DP 619, SALA 135668	1	Spain: Lérida, Espot	31TCH4215
Odontites vernus	DP 636, SALA 135687	2 (1^{2x})	Spain: Gerona, Ribes de Freser	31TDG3181
Odontites vernus	DP 638, SALA 135689	3	Spain: Gerona, Campdevanol	31TDG3176
Odontites vernus	DP 663, SALA 135713	3	Spain: Granada, Quéntar	30SVG6420
Odontites vernus	DP 683, SALA 135733	2	Spain: Teruel, Linares de Mora	30TYK0465
Odontites vernus	DP 694, SALA 135744	2	Spain: Valladolid, Aldeamayor de San Martín	30TUL5997
Odontites vernus	DP 696, SALA 135746	32D+36T (1^{2x}+1^{4x})	Spain: Valladolid, San Miguel del Arroyo	30TUL7888
Odontites vernus	DP 999, SALA 110023	1	Spain: Burgos, Contreras	30TVM6352
Odontites vernus	DP 1277, SALA 150522	30	Spain: Burgos, Tejada	30TVM5544
Odontites vernus	ER 7844, SALA 110695	1^{4x}	Spain: Huesca, Saravillo	31TBH7415
Odontites vernus	ER 7851, SALA 110696	3	Spain: Huesca, Bisaurri	31TBH9509
Odontites vernus	ER 7863, SALA 110693	2	Spain: Toledo, Tembleque	30SVJ4592
Odontites vernus	ER 7876, SALA 110709	1^{2x}	Spain: Almería, Fondón	30SWF1293
Odontites vernus	ER 7890, SALA 110730	2	Spain: Lugo, Samos	29TPH4631
Odontites vernus	ER 7971, SALA 135644	1	Spain: Orense, Castro Caldelas	29TPG3089
Odontites vernus	ER 8053, SALA 156498	1	Spain: Burgos, Encío	30TVN9224
Odontites vernus	G. Domina s.n., PAL 88463	1^{2x}	Italy: Sicilia, Geraci Siculo	33SVB2592
Odontites vernus	G. Tuleu s.n., CBFS 5135	2[f]	Czech Republic: South Bohemia, České Budějovice	33UVQ5925
Odontites vernus	LD 908, SALA 110700[e]	2	Spain: Valladolid, Aldeamayor de San Martín	30TUL6698
Odontites vernus	LD 910, SALA 110698	3	Spain: Valladolid, Canillas de Esgueva	30TVM0723
Odontites vernus	LD 931, SALA 110715	1	Spain: Soria, El Royo	30TWM3235
Odontites vernus	LD 944, SALA 110736	2	Spain: Burgos, Oña	30TVN8228
Odontites vernus	LD 979, SALA 110715[e]	2	Spain: Soria, El Royo	30TWM3235
Odontites vernus	MO 4522, SALA 135623	1^{4x}	Spain: Burgos, Merindad de Río Ubierna	30TVN4205
Odontites vernus	MO 5531, SALA 137348	2	Croatia: Lika-Senj, Plitvička Jezera National Park	33TWK5466
Odontites vernus	MO 5574, SALA 153253	1	Spain: Burgos, Frías	30TVN7635
Odontites vernus	MS 944, SALA 128791	1	Spain: Huesca, Ansó	30TXN8152
Odontites vernus	SA 415, SALA 137353	1	Macedonia: Kavadarci, betw. Ro den and Majden	34TEL7959
Odontites viscosus subsp. *asturicus* M. Laínz	DP 874, SALA 137373	2	Spain: León, Puebla de Lillo	30TUN0774
Odontites viscosus subsp. *australis* (Boiss.) Jahand. & Maire	DP 566, SALA 136267	1	Spain: Granada, Güéjar Sierra	30SVG5712
Odontites viscosus subsp. *australis*	VL 91, SALA 156301	1	Morocco: Chefchaouen, Jbel L'akraa	30SUD0490
Odontites viscosus subsp. *granatensis* (Boiss.) Bolliger	JPG ODOGRA-G01, SALA 135386[e]	1	Spain: Granada, Sierra Nevada	30SVG6207

Appendix 1. Continued.

Species	Collector no. and voucher accession[a,b]	*n*[c]	Collection locality	Coordinates[d]
Odontites viscosus subsp. *granatensis*	JPG 130, no voucher	1	Spain: Granada, Sierra Nevada	30SVG6208
Odontites viscosus subsp. *lusitanicus* Bolliger	MS 959, SALA 123308	1	Portugal: Estremadura, Sesimbra	29SMC8151
Odontites viscosus subsp. *lusitanicus*	MS 961, SALA 123309	1	Portugal: Estremadura, Sesimbra	29SMC8352
Odontites viscosus (L.) Clairv. subsp. *viscosus*	BR 165, SALA 142122	1	France: Provence-Alpes-Côte d'Azur, Marseille	31TFJ9705
Odontites viscosus subsp. *viscosus*	DP 616, SALA 135665	1	Spain: Lérida, Sarroca de Bellera	31TCG2492
Parentucellia latifolia (L.) Caruel	MO 6019, SALA 142077	2	Spain: Cáceres, Hervás	30TTK5659
Parentucellia viscosa (L.) Caruel	MO 6021, SALA 142079	2	Spain: Cáceres, betw. Guijo de Granadilla and Mohedas de Granadilla	29TQE3956

Note: *n* = number of individuals sampled.

[a]Abbreviations (collector numbers): AH = Alberto Herrero; AQ =Alejandro Quintanar; BR = Blanca Rojas-Andrés; CA = Carlos Aedo; DP = Daniel Pinto; ER = Enrique Rico; JPG = Julio Peñas de Giles; LD = Luis Delgado; MO = M. Montserrat Martínez-Ortega; MS = María Santos (except for MS 5056, SALA 125030, which refers to Miguel Sequeira); NLG = Noemí López González; SA = Santiago Andrés-Sánchez; SC = Santiago Castroviejo; VL = Victor Lucía.

[b]Herbarium specimens are lodged at the herbarium of Universidad de Salamanca (SALA), Salamanca, Spain; University of South Bohemia (CBFS), České Budějovice, Czech Republic; Universidad de Concepción (CONC), Concepción, Chile; Herbarium Mediterraneum Panormitanum (PAL), Palermo, Italy; and Real Jardín Botánico–Consejo Superior de Investigaciones Científicas (MA), Madrid, Spain. DNA samples are deposited at Biobanco de ADN Vegetal (Universidad de Salamanca), Salamanca, Spain.

[c]2*x*, 4*x* indicate ploidy level of individuals used in initial screening by agarose gel electrophoresis.

[d]Coordinates are in MGRS format and using WGS84 Datum.

[e]Silica gel–dried material and voucher specimen were collected in the same location but on different dates.

[f]Individuals used to obtain 454 sequence library.

APPENDIX 2

Appendix 2. Primers rejected and reasons for discarding.

Locus	Primer sequences (5′–3′)	Repeat motif	PCR product size (bp)	T_a (°C)	GenBank accession no.	Discarding reason
Ov-1	F: TCCTTAGAAGGACCCTCGAAAT R: TCAGTACATTTGTTACTTTTCAGCTA	$(AAT)_{11}$	93	—	KT777565	Inconsistent amplification
Ov-3	F: CTCTCCTTCATCACCCCTTCTT R: ACAAATTGAGAACCACTTTCCC	$(AC)_{11}$	124	54	KT777575	Genotyping difficulties
Ov-4	F: CACCTTTCTCATGAATCCATCT R: GTATGATGAAAATGGACGGGTT	$(AAAT)_9$	276	—	KT777576	Spurious bands in gel
Ov-7	F: GTCCGAAGCTCAAAGAGAAATC R: ACGTGAATAGATCTTCGACGGA	$(CCG)_7$	81	—	KT777584	Low levels of polymorphism in gel
Ov-8	F: TGCCGTTAAAGTCTCAGATCAA R: ATAATTTCACTAACGGCGAAGC	$(AC)_{10}$	103	—	KT777585	Low levels of polymorphism in gel
Ov-9	F: AATTCATAAGGCTGCTGCAGAT R: AATATCCATATGGTTTCAGCGG	$(AG)_{10}$	84	—	KT777586	Low levels of polymorphism in gel
Ov-11	F: GATTCATTGATTCGTTTATGTGT R: AATGCCACAACTTTGCATCTAA	$(AAC)_5$	99	—	KT777591	Low levels of polymorphism in gel
Ov-12	F: AAAGATCTGCAAACAAACAGCA R: GCATTATTCTCTATCCCACCCA	$(AC)_{13}$	105	55	KT777592	Genotyping difficulties
Ov-13	F: TAAGCATAAAACTGGAGGGGTC R: CGTTTGTCGAGCTTTATTTTCC	$(AC)_{10}$	108	—	—	Unsuccessful amplification
Ov-14	F: GCCACGTATGTTTAGCCTTGTA R: GCTTCTCTTTTGTGGGGTTTATT	$(AAT)_6$	161	—	—	Unsuccessful amplification
Ov-16	F: AGCTACCCAATATTCAGGGGAT R: ATGGAATACTCCTCCCTCCCT	$(AG)_8$	361	—	—	Unsuccessful amplification
Ov-18	F: CGTTCATCAACTTGACAAGAGC R: CAGAAGACCAACCAACTCTCCT	$(AG)_{22}$	179	—	—	Unsuccessful amplification
Ov-22	F: CAATTTAGGTCGAACTTGCACA R: GATATTCAGAATGACGGGAAGC	$(ACC)_5$	159	—	KT777614	Spurious bands in gel
Ov-23	F: ACTCCTTTCGTTGCCTATACCA R: AGATGTCGTACTCGCAAACAGT	$(AAT)_5$	82	—	KT777615	Low levels of polymorphism in gel
Ov-24	F: AGTTTTCAGCTCCACAGGTTGT R: CTTGAAATTGGTTCTGGAAAGG	$(ACC)_5$	89	—	KT777616	Low levels of polymorphism in gel
Ov-26	F: AAGGAGCTGATGAAAGCAGTTT R: AGCTCATATTCTCCGGGTTACA	$(AC)_5$	170	55	KT777621	Monomorphic
Ov-27	F: CTCAGTGTAGTTCCGTCATTGC R: GCAATTCACAAATTCAATCCAA	$(AG)_6$	276	—	—	Unsuccessful amplification
Ov-29	F: GTACCCATATTTTTCCACCACG R: ATGGAATACTCCTCCCTCCCT	$(AG)_8$	275	—	—	Unsuccessful amplification
Ov-31	F: TGGGAGTAGGGTAATCAAAGGA R: AGAAGACCAACCAACTCTCCTG	$(AG)_{22}$	225	—	—	Unsuccessful amplification
Ov-32	F: GATCCATTAGCAATGGGACTTT R: TCGAGGAGATGTAATGGTTTTG	$(AG)_{11}$	411	53	KT777630	Genotyping difficulties
Ov-34	F: CGCATTTCACGAATCAAACTAA R: AGCCTTGTAGCAGAAGCATTTC	$(AC)_5$	208	—	—	Unsuccessful amplification
Ov-36	F: AATTCATCCTAGCGTGTTCCAT R: ACTTGGTTGGGATACGTTTAGC	$(AT)_5$	338	—	—	Unsuccessful amplification

Note: — = no information available; T_a = optimal annealing temperature.

Capítulo 6: *Macrosyringion longiflorum* (Lam.) Rothm. en el Norte de Marruecos[2]

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[2] Este capítulo fue publicado en una revista científica antes que el capítulo 2, de modo que aun considerábamos que *Macrosyringion* era un género separado de *Odontites.*

ABSTRACT

Macrosyringion longiflorum (Lam.) Rothm. (Orobanchaceae), a plant restricted to the Iberian Peninsula and Morocco, is cited for the second time in Northern Morocco. It has been located on the Talassemtane National Park (Western Rif) almost a century after Mouret collected it in Anoceur (Middle Atlas). This new population is halfway between those present in the South of the Iberian Peninsula and that of the Middle Atlas.

Keywords: *Macrosyringion longiflorum*; Morocco; Orobanchaceae; Talassemtane National Park.

RESUMEN

Se cita por segunda vez en el Norte de Marruecos *Macrosyringion longiflorum* (Lam.) Rothm. (Orobanchaceae), planta de distribución restringida a la Península Ibérica y Marruecos. Ha sido localizada en el Parque Nacional Talassemtane (Rif Occidental) tras casi un siglo desde que Mouret la recolectara en Anoceur (Atlas Medio). Esta nueva población se sitúa a medio camino entre las presentes en el Sur de la Península Ibérica y la del Atlas Medio.

Palabras clave: *Macrosyringion longiflorum*; Marruecos; Orobanchaceae; Parque Nacional Talassemtane.

Dentro del pequeño género *Macrosyringion* Rothm., desgajado de *Odontites* Ludw. por características de la corola (Rothmaler, 1943) y de la ornamentación del polen (Bolliger & Wick, 1990), se incluyen sólo dos especies que se distribuyen por los dos extremos de la región mediterránea: *M. glutinosum* (M. Bieb.) Rothm., fundamentalmente por el Mediterráneo oriental (Bolliger, 1996), y *M. longiflorum* (Lam.) Rothm. por el extremo occidental de dicha región (Bolliger, l996; Rico, 2009).

Macrosyringion longiflorum se conoce casi exclusivamente de la Península Ibérica, donde es bastante frecuente, en concreto de la mitad oriental y de parte del NW de la misma (Rico, 2009). Fuera de esa península, únicamente se conocía de dos localidades, una de zonas limítrofes del S de Francia, en la vertiente septentrional del Pirineo Oriental (Bouchard 1991), y la otra del N de África.

En el continente africano al parecer sólo había sido herborizada en una ocasión, hace ya casi 100 años, por Mouret en 1913 en Anoceur, en el Atlas Medio marroquí, como indican tanto el monógrafo del género *Odontites* s.l., Bolliger (1996), quien revisó el material correspondiente depositado en los herbarios G y MPU, como Ibn Tattou (2007) en la flora marroquí más reciente.

La nueva localidad marroquí que aportamos se encuentra en las montañas de Rif:

MARRUECOS: Rif occidental, Bab Taza, Jbel Lakraa, 30SUD0490 Datum WGS 84, 1832 m, claros de bosque mixto de cedros y pinsapos, 10-VII-2010, V. Lucía (VL 82), D. Pinto Carrasco, E. Rico & T. Romero, SALA 137638 (Ejemplares vegetativos, aún sólo con hojas).

En esa localidad fue observada por primera vez y fotografiada en floración (fig. 1) por uno de nosotros, S. Doglio, el 30 de octubre de 2009, junto a la pista próxima a la casa forestal del Parque Nacional Talassemtane; buscada de nuevo en otoño de 2010 en el mismo lugar, no fue encontrada. Sin embargo, en julio de 2010 encontramos algunos ejemplares, aún pequeños y sólo en hojas (fig. 2) que pasaban bastante desapercibidos, pero inconfundibles, en un lugar muy próximo de la base de la falda NW del Jbel Lakraa. En esta última fecha se recorrió gran parte del macizo pero no se vieron más ejemplares.

Figura 1: *Macrosyringion longiflorum*, detalle de la inflorescencia (Jbel Lakraa, Bab Taza)

Figura 2. *Macrosyringion longiflorum*, individuo joven en hojas (Jbel Lakraa, Bab Taza)

El Jbel Lakraa, y todo el conjunto del P.N. Talassemtane, ha sido una de las zonas de Marruecos más visitadas tanto por botánicos de inicios o mediados del siglo pasado, de donde incluso publicaron algún listado de plantas pormenorizado (Font Quer, 1931), como por diversos botánicos marroquíes y españoles en los últimos años, sobre todo en relación con la elaboración del completo catálogo de las plantas vasculares del N de Marruecos (Valdés *et al*., 2002) o el del catálogo más reciente y específico del Rif (Mateos & Valdés, 2010).

Por ello sorprende un poco que la planta no fuera recolectada con anterioridad; la explicación estaría en su floración muy tardía (de mediados de verano a mediados de otoño), la dificultad de diferenciarla en estado vegetativo de ejemplares de otros *Odontites* con los que convive y, por lo que hemos podido constatar en los dos últimos años, su extraordinaria escasez en el macizo.

Los ejemplares observados crecían en los pastos ralos de terófitos desarrollados sobre las repisas de roquedos o los cascajares de las calizas del macizo, en las zonas abiertas y soleadas del bosque de mixto de *Abies marocana* y *Cedrus atlantica*. Entre las especies acompañantes más abundantes se encontraban *Odontites viscosus* subsp. *australis, Astragalus armatus, Ononis pusilla, Bupleurum fruticescens* subsp. *spinosum, Pimpinella tragium, Acinos alpinus, Campanula mollis, Chiliadenus glutinosus o Santolina rosmarinifolia.*

El hábitat de la población rifeña es similar al de muchas de las zonas donde crece en la Península Ibérica (Rico, 2009) y, además, se encuentra muy bien representado en las zonas de substrato básico del N de Marruecos. Sin embargo, el hábitat donde Mouret la recolectó en Anoceur es algo diferente: "coteaux sablonneux" (cf. Bolliger, 1996) o "pentes sablonneuses" (cf. Ibn Tattou, 2007). Durante julio de 2010 visitamos los alrededores de Anoceur y no vimos ningún lugar con "pendientes arenosas"; no obstante, buscamos la planta en algunos pastos sobre calizas de la zona sin ningún resultado positivo.

Por tanto, esta especie parece ser extremadamente rara en el Norte de África. En relación con su escasez (solo una población conocida en aquel momento) es recogida en el catálogo de las plantas raras o amenazadas de Marruecos de Fennane & Ibn Tattou (1998).

La población que damos a conocer ahora dista, en línea recta, unos 185 km de las poblaciones septentrionales más cercanas, las cordobesas y malagueñas del S de la Península Ibérica. La otra población marroquí se encuentra a unos 165 km en línea recta hacia el sur. La población rifeña permite reducir aproximadamente a la mitad el enorme hiato que existía entre la aislada población del Atlas Medio y el área amplia, y casi endémica, de la Península Ibérica. Aun así, la distancia entre las poblaciones africanas entre sí y con las ibéricas sigue siendo demasiado grande como para que pueda ser franqueada de forma habitual por la diáspora de *M. longiflorum*, ya que dispersa sus semillas por caída simple, aunque debido al escaso peso de las mismas quizá también podrían ser llevadas por el viento. Por tanto, es probable que su presencia en esas poblaciones disyuntas norteafricanas se deba a un hecho puntual de dispersión a larga distancia a través del estrecho de Gibraltar. Este fenómeno ya ha sido puesto de manifiesto de forma fehaciente mediante el uso de herramientas moleculares en otras plantas que tampoco presentan mecanismos especiales de dispersión a larga distancia, como *Cistus ladanifer* (Guzmán & Vargas, 2009). No hay que descartar la posibilidad de una reducción de un área amplia y continua anterior, pero parece poco probable, sobre todo si tenemos en cuenta la ausencia de hábitat favorables en extremo S de la Península Ibérica, por el predominio de sustratos ácidos en zonas de la provincia de Cádiz.

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Capítulo 8: Conclusiones

EN CASTELLANO

1. Se ha realizado un estudio filogenético de la tribu Rhinantheae (Orobanchaceae) y centrado en el género *Odontites*, sobre la base de secuencias de ADN nucleares (ITS) y plastidiales (región *trnK* e intrón de *rps16*). El análisis de estos datos demuestra que el género *Odontites* es monofilético cuando se incluyen de él las especies que Rothmaler y Bolliger segregaron en los géneros *Macrosyringion*, *Bornmuellerantha* y *Bartsiella*. Sin embargo, el género *Odontitella* es más cercano a *Nothobartsia* y se mantiene desligado de *Odontites*.

2. Como ya indicaban algunos estudios filogenéticos anteriores, el género *Phtheirospermum* es polifilético. La especie tipo del género, *Ph. japonicum*, es cercana al género *Pedicularis* (tribu Pedicularideae), mientras que las otras tres especies de *Phtheirospermum* forman un clado junto con *Pterygiella*. Para evitar la polifilia, se han propuesto 3 combinaciones nomenclaturales para incluir esas tres especies en *Pterygiella*: *Pt. muliensis* (C.Y.Wu & D.D.Tao) Pinto-Carrasco, E.Rico & M.M.Mart.Ort., comb. nov.; *Pt. parishii* (Hook.f.) Pinto-Carrasco, E.Rico & M.M. Mart.Ort., comb. nov.; y *Pt. tenuisecta* (Bureau & Franch.) Pinto-Carrasco, E.Rico & M.M.Mart.Ort., comb. nov.

3. Dentro del género *Odontites*, se han detectado 5 linajes genéticos que son morfológicamente coherentes. Se han detectado sinapomorfías morfológicas para los linajes K.2-Macrosyringion (p.ej., tubo de la corola largo), K.3-Bornmuellerantha (p.ej., corola subrotada) y K.4-Viscosus (p.ej., labio superior de la corola doblado hacia abajo). Sin embargo, no se han encontrado sinapomorfías morfológicas para los linajes K.1-Pyrenaeus y K.5-Vernus, pero se pueden definir mediante conjuntos de caracteres. Para diferenciarlos, la presencia (K.1-Pyrenaeus) o ausencia (K.5-Vernus) de pelos glandulares en el cáliz es especialmente útil.

4. Dentro del linaje K.5-Vernus, *O. luteus* y *O. lanceolatus* no están genéticamente bien diferenciadas, y morfológicamente son muy similares. Por ello, proponemos que las dos subespecies de *O. lanceolatus* (*O. lanceolatus* subsp. *lanceolatus* y *O. lanceolatus* subsp. *provincialis*) sean incluidas en la variabilidad de *O. luteus* con rango de subespecie. Una situación similar se presenta entre las especies *O. vernus*, *O. vulgaris* y *O. litoralis*. En este caso, para incluir las subespecies de *O. vulgaris* y *O. litoralis* dentro de *O. vernus* con rango de subespecie, es necesario proponer las siguientes combinaciones nomenclaturales: *O. vernus* subsp. *fennicus* (Markl.) Pinto-Carrasco, E.Rico & M.M.Mart.Ort., comb. nov.; *O. vernus* subsp. *himalayicus* (Pennell) Pinto-Carrasco, E.Rico & M.M.Mart.Ort., comb. nov.; y *O. vernus* subsp. *mesatlanticus* (Emb. & Maire) Pinto-Carrasco, E.Rico & M.M.Mart.Ort., comb. nov.

5. Las muestras de *Odontites* recolectadas en La Palma (Islas Canarias) son genéticamente indistinguibles de las del endemismo madeirense *O. hollianus*. Existe una sutil diferencia morfológica entre ambas poblaciones en la longitud de las papilas de los filamentos de los estambres, pero no es de suficiente calado para reconocer dos entidades taxonómicas diferentes. Por tanto, *O. hollianus* es un endemismo macaronésico.

6. Se han estudiado las especies del grupo de *O. vernus* (*O. vernus*, *O. kaliformis* y *O. recordonii*) presentes en la Península Ibérica por medio de marcadores hipervariables de tipo AFLP, utilizando el resto de las especies ibéricas de *Odontites* y *Odontitella* como grupos externos. Las relaciones filogenéticas obtenidas mediante AFLPs son similares a las que se derivan de las secuencias de ADN nucleares y plastidiales. Las tres especies del grupo son monofiléticas, y no se detecta flujo genético entre las mismas. Destaca la baja diversidad genética del endemismo levantino *O. kaliformis*, que se encuentra en peligro de extinción (EN A2ac; B1ab(iii,iv)+2ab(iii,iv)).

7. En el set de datos de AFLPs de *O. recordoni*, se detecta una estructuración genética clara a nivel de población. A un nivel jerárquico superior, se obtienen tres grupos genéticos poco diferenciados entre sí, observándose poblaciones con una composición alélica intermedia entre cada par de grupos. Estos tres grupos genéticos tienen una distribución geográfica coherente con las condiciones bioclimáticas: Cluster A en las partes altas de la cuenca del rio Ebro (piso supramediterráneo); Cluster B en el sur de la distribución de la especie (piso termomediterráneo) y, de manera disjunta, en Guadalajara (por una posible dispersión a larga distancia); y Cluster C principalmente en las partes bajas de la cuenca del rio Ebro (piso mesomediterráneo).

8. Se han realizado modelos de distribución potencial para cada grupo genético de *O. recordonii*, tanto a tiempo actual como proyectado a tres momentos del pasado climatológicamente muy diferentes (Holoceno Medio, LGM y LIG). Esto permite detectar áreas de clima estable a lo largo del tiempo que han podido servir de refugio a cada grupo genético: Cluster A en Nordeste de Castilla y León y Sur del País Vasco; Cluster B en la provincia de Alicante; y Cluster C en torno a la frontera entre La Rioja, Navarra y País Vasco.

9. De los 456 alelos obtenidos para *O. recordonii* mediante AFLPs, 81 muestran signos de selección natural. Además, 58 alelos están correlacionados con alguna variable ambiental. Las variables ambientales correlacionadas con los alelos que sustentan la diferenciación entre los tres grupos genéticos son, principalmente, aquellas relacionadas con la estacionalidad de las temperaturas y las precipitaciones, y la severidad de la sequía estival, aspectos clave en el ciclo de vida de las plantas en un clima mediterráneo.

10. Se ha desarrollado un nuevo set de 14 marcadores microsatélite polimórficos para *O. vernus* a partir de una librería de secuencias enriquecidas en motivos microsatélite, generada mediante secuenciación de nueva generación. Estos marcadores se han testado satisfactoriamente en poblaciones procedentes de diversos países europeos, y tanto en individuos diploides como tetraploides.

11. Se ha probado la amplificación cruzada de 18 marcadores microsatélite (incluidos los 14 seleccionados para *O. vernus*) en 30 taxones diferentes (21 de *Odontites* y 9 de otros géneros de la tribu Rhinantheae). Los resultados obtenidos son muy diversos, detectándose más amplificaciones exitosas en especies filogenéticamente cercanas a *O. vernus* (p.ej., *O. recordonii*, *O. kaliformis*, *O. hollianus*, *O. luteus* y *O. corsicus*).

12. Se ha estudiado la variabilidad genética y la ploidía en 100 poblaciones de *O. vernus* por medio de secuencias de ADN plastidial (herencia materna), marcadores microsatélite (herencia biparental), y estimaciones de nivel de ploidía usando citometría de flujo. De los 301 individuos analizados, se ha estimado que 129 son diploides (42,9%) y 172 (57,1%) son tetraploides, y no se ha detectado ningún individuo triploide. La distribución de los dos citotipos en la Península Ibérica es parapátrica en mosaico, y solo se ha detectado que convivan en dos poblaciones. Sobre la base de las frecuencias de alelos compartidos entre los dos citotipos y de alelos privados del citotipo tetraploide, es probable que el citotipo tetraploide se haya generado por autopoliploidización al menos en dos ocasiones.

13. Se han detectado 20 haplotipos de ADN plastidial en *Odontites vernus*, que se estructuran en dos haplogrupos bien diferenciados. Uno de ellos está compuesto mayoritariamente por individuos diploides (75%) y el otro mayoritariamente por tetraploides (81%). Geográficamente, la distribución de ambos haplogrupos se solapa ampliamente, excepto en el Sur de la Península Ibérica donde solo se han encontrado individuos diploides pertenecientes al haplogrupo diploide.

14. Los marcadores genéticos de herencia biparental (SSRs) analizados en *O. vernus* muestran una estructuración más compleja que los de herencia materna. Si se usan métodos de distancia genética, se obtienen 4 grupos (2 integrados por individuos diploides, y otros 2 por tetraploides), mientras que, si se usan métodos bayesianos de análisis de estructura genética, se hallan 7. Se detecta un escaso flujo genético entre grupos, a excepción de en las poblaciones 12 y 69.

15. Los 7 subgrupos genéticos detectados dentro de la variabilidad de *O. vernus* en la Península Ibérica tienen distribuciones geográficas continuas, que coinciden con áreas que se considera que han servido de refugio para la fauna y la flora ibéricas en el contexto de las oscilaciones climáticas del Cuaternario. Los refugios que se proponen para cada grupo son: Cuenca del Ebro para K7.1; Norte de Portugal y/o Sistema Central para K7.2 y K7.3; Pirineos para K7.4 y K7.5; Cuenca del Ebro, Pirineos y/o Cordillera Cantábrica para K7.6; y Sierras Béticas para K7.7. Por tanto, estos resultados apoyan la hipótesis de los "refugios dentro de refugios", que cada vez cuenta con un cuerpo de evidencia más amplio.

16. Durante las prospecciones realizadas en el Norte de Marruecos para recolectar las muestras utilizadas en esta tesis doctoral, se encontró una población de *Odontites longiflorus* en el Parque Nacional Talassemtane (Rif Occidental). Esto supone el redescubrimiento de esta especie tras casi un siglo desde que Mouret la recolectara por primera y única vez en el Atlas Medio. Esta nueva población se sitúa a medio camino entre las presentes en el Sur de la Península Ibérica y la del Atlas Medio.

IN ENGLISH

1. A comprehensive phylogenetic analysis of tribe Rhinantheae focused on *Odontites* and based on plastid (*trnK* region and *rps16* intron) and nuclear DNA (ITS) is presented. This analysis demonstrated that *Odontites* is monophyletic when it is circumscribed to include the species of the genera *Macrosyringion*, *Bornmuellerantha* and *Bartsiella*. But *Odontitella*, which was shown to be phylogenetically close to *Nothobartsia*, should be excluded from *Odontites.*

2. As indicated by previous phylogenetic studies, the Asian genus *Phtheirospermum* is regarded as polyphyletic. The type species *Ph. japonicum* was shown to be phylogenetically close to *Pedicularis* (tribe Pedicularideae), while the remaining species of *Phtheirospermum* were recovered in a monophyletic clade together with *Pterygiella*. To avoid polyphyly, three species of *Phtheirospermum* must be transferred to *Pterygiella* and the following three new nomenclatural corresponding combinations have been proposed for them: *Pt. muliensis* (C.Y.Wu & D.D.Tao) Pinto-Carrasco, E.Rico & M.M.Mart.Ort., comb. nov.; *Pt. parishii* (Hook.f.) Pinto-Carrasco, E.Rico & M.M. Mart.Ort., comb. nov.; y *Pt. tenuisecta* (Bureau & Franch.) Pinto-Carrasco, E.Rico & M.M.Mart.Ort., comb. nov.

3. Five morphologically distinct lineages were identified within *Odontites*. Morphological synapomorphies were found for lineages K.2-Macrosyringion (e.g., long corolla tube), K.3-Bornmuellerantha (e.g., subrotate corolla) and K.4-Viscosus (e.g., corolla upper lip folded downwards). Although no morphological synapomorphies were encountered for lineages K.1-Pyrenaeus and K.5-Vernus, they can be easily identified by a combination of traits. To this aim, the long-stalked glandular hairs present (K.1-Pyrenaeus) or absent (K.5-Vernus) from the calyx are particularly useful.

4. Within lineage K.5-Vernus, *O. luteus* and *O. lanceolatus*, which are morphologically similar did not appear to be genetically distinct. Therefore, it is here proposed that the traditionally accepted subspecies of *O. lanceolatus* (i.e., *O. lanceolatus* subsp. *lanceolatus* and *O. lanceolatus* subsp. *provincialis*) are transferred to *O. luteus* preserving the subspecific rank. A similar situation was found for *O. vernus, O. vulgaris* and *O. litoralis*. In this case, to include the subspecies described under *O. vulgaris* and *O. litoralis* within the variability of *O. vernus*, the following three combinations have been proposed: *O. vernus* subsp. *fennicus* (Markl.) Pinto-Carrasco, E.Rico & M.M.Mart.Ort., comb. nov.; *O. vernus* subsp. *himalayicus* (Pennell) Pinto-Carrasco, E.Rico & M.M.Mart.Ort., comb. nov.; and *O. vernus* subsp. *mesatlanticus* (Emb. & Maire) Pinto-Carrasco, E.Rico & M.M.Mart.Ort., comb. nov.

5. The samples of *Odontites* collected in La Palma (Canary Islands) were not genetically distinct from those assigned to the Madeiran endemic species *O. hollianus*. Although the length of the papillae on the stamen filaments is slightly different between the individuals present in both Archipelagos, this incipient morphological difference does not sufficiently justify the recognition of the Canarian plants as a new taxon. Therefore, *O. hollianus* would be regarded as a Macaronesian endemic.

6. To try to shed additional light into the phylogenetic relationships underlying among the species included within the *O. vernus* group represented in the Iberian Peninsula (*O. vernus, O. kaliformis* y *O. recordonii*), we used AFLPs as hypervariable DNA markers and the remaining Iberian species of *Odontites* and *Odontitella* as outgroups. The obtained results confirm those obtained based on nuclear and plastid DNA sequencing. The three species that conform the group appeared as monophyletic and gene flow among them was not detected. The eastern Iberian endemic *O. kaliformis* –that is catalogued as an endangered species (EN A2ac; B1ab(iii,iv)+2ab(iii,iv))– showed very low genetic diversity.

7. Regarding the also endemic *O. recordonii*, the AFLP markers used revealed clear structure of the genetic variability at the population level. Additionally, three poorly differentiated genetic groups clustering several populations were found, although some populations show genetic admixture between different pairs of these genetic groups. These three higher-level genetic groups displayed distributions in accordance with bioclimatic conditions; thus, Cluster A was present in the Upper Ebro Basin (supramediterranean belt), Cluster B was distributed throughout the southern part of the distribution area of the species (thermomediterranean belt) as well as in Guadalajara province (probably as a consequence of long-distance dispersal), and Cluster C was mainly represented in the Lower Ebro Basin (mesomediterranean belt).

8. For every genetic cluster detected within *O. recordonii*, ecological niche models were performed at present time and at three different time frames, i.e., mid-Holocene (6,000 years before present, YBP), last glacial maximum (LGM, 21,000 YBP) and Last Interglacial period (LIG, ca. 120,000-140,000 YBP). Thus, the following areas of probable persistence along time (putative refugia) were found: Cluster A in north-eastern Castile and Leon and southern Basque Country; Cluster B in Alicante province; and Cluster C at the area bordering La Rioja, Navarra and the Basque Country.

9. According to the analyses performed, 81 out of 456 alleles obtained for *O. recordonii* could be under the effects of natural selection at a regional and/or local level. Additionally, 58 alleles showed significant correlations with any environmental variable. The environmental variables correlated with those alleles that significantly contribute to the differentiation among the three genetic clusters found within *O. recordonii* were associated to temperature and precipitation seasonality, as well as to the intensity of summer drought, all of them parameters of capital importance for plants in Mediterranean type climates.

10. Fourteen polymorphic and reproducible microsatellite loci were identified and optimized from *O. vernus* using a microsatellite-enriched library and Next Generation Sequencing platform. The utility of this set of primers was tested in populations from different European countries and in diploid and tetraploid individuals.

11. Transferability of a set of 18 primers corresponding to SSRs loci (including the just referred 14 selected for *O. vernus*) was tested in 30 taxa (21 belonging to *Odontites* and 9 from other genera of the tribe Rhinantheae). The obtained results were diverse, with more successful amplifications corresponding to taxa phylogenetically close to *O. vernus* (e.g., *O. recordonii*, *O. kaliformis*, *O. hollianus*, *O. luteus* and *O. corsicus*).

12. Twelve microsatellites optimized from *O. vernus* were used, together with nuclear and plastid DNA sequences, as well as flow cytometry, to investigate the genetic variability and ploidy levels of 100 Iberian populations of this species. One hundred and twenty-nine out of 301 individuals studied were shown to be diploids (42,9%), while 172 (57,1%) were tetraploids, and no triploid was detected. The diploid and tetraploid cytotypes were shown to be distributed following a mosaic parapatry model and only two mixed-ploidy population was discovered. Considering the frequencies of the alleles shared by both cytotypes, as well as that of the private alleles corresponding to the tetraploid cytotype, it seems likely that this cytotype was originated, at least twice independently, through autopolyploidization.

13. Within *O. vernus*, 20 cpDNA haplotypes were detected, which were found to be structured into two well differentiated haplogroups. One of them is mainly composed of diploid individuals (75%), while the other one is predominantly formed by tetraploids (81%). The geographic distribution of both haplogroups showed extensive overlapping, except in the south of the Iberian Peninsula, where only diploid individuals from the diploid haplogroup were found.

14. The biparentally inherited SSR markers analysed in *O. vernus* showed more complex structure than that revealed by the maternally inherited DNA markers. The genetic distance-based methods detected 4 groups (2 composed of diploids and the remainder 2 formed by tetraploids), but the Bayesian analysis of population structure found 7 genetic groups. Only scarce levels of gene flow among groups are detected, except for populations 12 and 69.

15. The 7 just referred genetic groups found within the variation of the Iberian *O. vernus* show distributions that coincide with areas reputed to have represented refugia within the Iberian Peninsula during the climatic oscillations of the Quaternary. The refugia proposed for each group were: Ebro Basin for K7.1; North of Portugal and/or Central System for K7.2 and K7.3; Pyrenees for K7.4 and K7.5; Ebro Basin, Pyrenees and/or Cantabrian Range for K7.6; and Betic Ranges for K7.7. The obtained results give additional support to the "refugia within refugia" hypothesis.

16. During the surveys carried out in the North of Morocco to collect the samples used in this doctoral thesis, a population of *O. longiflorus* was found in the Talassemtane National Park (Western Rif). This represents the rediscovery of this species in North Africa almost a century after Mouret collected it for the first and only time in the Middle Atlas. This new population is situated halfway between those present in the South of the Iberian Peninsula and that of the Middle Atlas.