



Article

On the morphological, biological and genetic heterogeneity of the genus *Orchis* (Orchidaceae, Orchidinae)

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Abstract

Circumscription of *Orchis* (Orchidaceae, Orchidinae) has undergone many changes in the last few decades. However, delimitation of the genus is still subject to debate. In its present state, *Orchis* s.str. appears to be composed of two well-differentiated, monophyletic groups of species, now treated as subgenera (*Orchis* s.s. and *Masculae*). Here we present detailed morphological measurements of floral and vegetative traits collected for a large number of species of the two subgenera and the related genus *Anacamptis* to provide further insights in the taxonomy of this genus. Using multivariate analyses, we show that three groups of species are clearly separated morphologically and that this separation is more pronounced between the two subgenera than between *Masculae* and *Anacamptis*. This morphological variation is also supported by significant differences in seed micromorphology of the two subgenera. Review of the literature further provided significant differences in pollinator assemblages between the two subgenera, suggesting substantial pre-zygotic reproductive isolation, and a significant difference in diversity of fungal associates with species of the two subgenera, possibly contributing to post-zygotic reproductive isolation. The fact that no viable hybrid between the two subgenera has ever been observed under artificial or natural conditions is in line with these findings. Based on these findings, an appropriate taxonomic rank for the two groups is discussed.

Key words: generic delimitation, *Masculae*, morphometrics, orchid phylogenetics, reproductive isolation

Introduction

Orchis Linnaeus (1753: 939) has undergone many changes in circumscription since it was first described. Several genera were separated from *Orchis* in previous decades, including genera now widely accepted, such as *Dactylorhiza* Necker ex Nevski (1937: 332), *Gymnadenia* Brown (1813: 191) and *Anacamptis* Richard (1817: 25, 33). Until recently, *Orchis* consisted of an aggregate of several groups of species with diverse morphology and different chromosome numbers (i.e., sections *Platycheilae* Klein 2004: 642, with $2n = 36$; sections *Dipterocheilae* Klein 2004: 643, *Orchis* and *Galericulatae* Klein 2004: 645, with $2n = 40, 42$). Additionally, it was widely recognised that hybrids occur between species within a group, but not between species of different groups (e.g. Klein 2004, Tyteca & Klein 2008).

A significant breakthrough was achieved with the advent of molecular phylogenetics (Bateman *et al.* 1997, Pridgeon *et al.* 1997). In analyses of a large number of species of the aforementioned groups, it appeared that *Orchis* as it had been considered until that time was paraphyletic, and monophyly of the aforementioned groups was recognised. As a consequence, species belonging to the former genus *Orchis* were redistributed into three genera, i.e., *Anacamptis*, *Neotinea* Reichenbach (1852: 29) and a residual genus *Orchis* (Bateman *et al.* 1997, Pridgeon *et al.* 1997). The last, however, still comprised representatives of two of Klein's sections, i.e. *Dipterocheilae* or the *O. mascula* (Linnaeus, 1753: 941) Linnaeus (1755: 310) group