

# Outline analysis for identifying *Limodorum* species from seeds

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**Abstract** — *Limodorum trabutianum* Batt. is an orchid species of the Italian flora, with a central-western stenomediterranean distribution, that is sporadic in the western part of the distribution area of the more common *L. abortivum* (L.) Sw., an eurimediterranean species. It occurs in Italy only with a few populations in Tuscany, Latium, Umbria, Sicily and Sardinia [1], often with *L. abortivum* [2], [3], [4] from which it is easily recognizable only during anthesis for the denser inflorescence spike, the ribbon-like lip without differentiation in epychile and hypochile, and for the spur that is very short or absent [5]. On the contrary, the identification of these two *taxa* during the fruiting phase is rather difficult or even impossible. The aim of this study is to verify the taxonomic value of *Limodorum* seeds, particularly of their shape, as highlighted from recent studies for other orchids [6], [7], in order to establish its usefulness for recognizing the two species.

We have identified 5 Italian populations of the two *taxa*: 2 populations of *L. trabutianum*, one within the Marturanum Regional Park (Barbarano Romano, Viterbo), the other near Cortona (Arezzo), and 3 populations of *L. abortivum*, near S. Martino al Cimino (Viterbo), in the M. Casoli Reserve (Bomarzo, Viterbo), and in the same site of *L. trabutianum* within the Marturanum Park. The phenology of these populations was monitored to collect mature seeds from naturally dehiscing capsules. The intra- and interspecific variability of seed shapes was analyzed with the methodology of Elliptic Fourier descriptors [8], which allows to describe in terms of harmonics each two-dimensional shape with a closed outline. For this outline analysis we used the software package SHAPE 1.3 [9]. An average of 100 seeds from each species and from each site was photographed with a NIKON Coolpix 5000 camera mounted on a LEITZ-ARISTOPLAN microscope, obtaining 500 digital images with a resolution of 300 dpi and a size of 800 x 1000 pixels. All images were prepared using Adobe Photoshop 7.0: as a first step, every foreign element was eliminated from the picture, thereby isolating the single seed, then its contrast with the background was maximized, and finally all images were saved in .bps format (24bit). The color images were converted

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to binary with *Chain Coder* before tracing the outlines in Chain-code, a coding system that describes the geometrical information of the shapes. Then the Chain-code file was transformed into a Normalized Elliptic Fourier file using *Chc2Nef* using 20 harmonics. The matrix of the harmonic coefficients underwent a process of data normalization based on the first harmonic, to transform the data into shape variables. Subsequently, a PCA was performed on the variance-covariance matrix of normalized coefficients using *PrinComp*, which gives a graphical output of the principal components (average shape  $\pm$  standard deviations).

The first results of the outline analysis confirm a low intraspecific variability of seed shape, but show a very high interspecific variability: *L. abortivum* seeds are very elongated, from fusiform to filiform, while *L. trabutianum* seeds are much wider and have a very lower length/width ratio. These results allow to distinguish between these two species even during the fruiting phase, simply using seed shape as a diagnostic character, avoiding the use of traditional morphometric analysis which need microscopic measurements.

**Index Terms** — image analysis, orchids, identification, plants.



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