



Phylogenetics of *Pecten* scallops based on three mitochondrial genes

Carlos Saavedra & Juan B. Peña



Instituto de Acuicultura de Torre la Sal (IATS-CSIC), 12595 Ribera de Cabanes (Castellón) SPAIN.

E-mail: saavedra@iats.csic.es

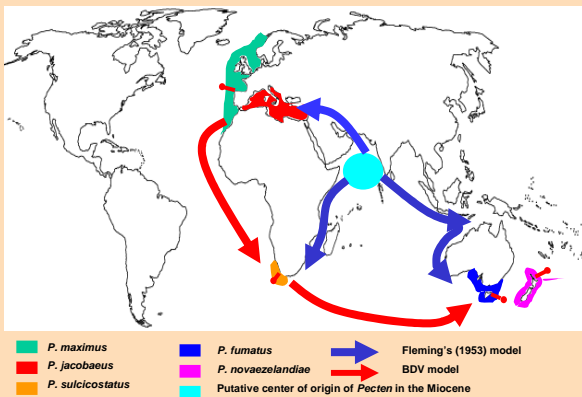
1. The problem of *Pecten* phylogeny

The scallops of the genus *Pecten* live in marine bottoms along the coasts of Europe, Africa, Asia and Australia and their neighbor islands. Current taxonomy is based on the morphology of the shell, especially on the sculpture. These characters have been used to separate two scallop species in Europe: *Pecten maximus* in the Atlantic and *P. jacobaeus* in the Mediterranean (Fig. 1). However, allozymes and DNA studies have shown that the genetic differentiation between these two taxa is not large, and more typical of different populations of the same species, rather than of separate species. This led to some authors to propose that the two European scallop forms should be considered as races or subspecies^{1,2}.

These results had implications for the taxonomy of the remaining *Pecten* species. If differences in shell shape and sculpture always reflected a slight genetic differentiation, as in the case of *P. maximus* and *P. jacobaeus*, all *Pecten* species could be viewed as a single, polytypic species with an almost worldwide distribution. While this is probably an extreme point of view, a clarification of these issues can be greatly helped by genetic analysis of populations.

This considerations apply especially to southern Australasian scallops. Morphology and allozymes indicate that there exist three *Pecten* species in the southern Australian continent and neighbour islands (*P. modestus*, *P. fumatus* and *P. novaezelandiae*)^{3,4}. In spite of living in opposite parts of the world (Fig. 2), morphology suggests a close phylogenetic relationship of these species with the European *Pecten* (Fig. 1). All five cited species have been included in a "jacobaeus" supraspecific group⁵.

Fig. 2. Distribution of the studied species, sampling sites, and paleo-routes of dispersal.



2. Alternative models of origin of southern Australasian *Pecten* scallops

In a previous study⁵, we analysed the phylogenetic relationship among south Australasian and northern Atlantic scallops by studying a ca. 550 bp of the 16S ribosomal RNA gene. We showed that the southern Australasian species *P. fumatus* and *P. novaezelandiae* were clearly separated from Atlantic *P. maximus* and Mediterranean *P. jacobaeus*. However, divergence between the Atlantic/Mediterranean clade and the southern Australasian clades was too small to fit the accepted model of separation at the time of the Tethys Sea closure (ca. 15 MY before present), proposed by Fleming⁶.

We proposed an alternative model based in Vermelij (1992)⁷ hypothesis of pre-Pleistocene trans-equatorial dispersal of *Pecten* from Europe to south Africa at the end of the Pliocene, and Beu and Darragh⁸ hypothesis of subsequent transportation from south Africa to Australia and New Zealand by the circum-Antarctic current to give rise to present-day *Pecten* south Australasian king scallop species. This "Beu-Darragh-Vermelij (BDV)" model predicts that the south African species *P. sulciostatus* should show an intermediate position in phylogenetic trees. We tested this prediction by partially sequencing 3 mitochondrial genes (12S and 16S ribosomal RNA, and cytochrome oxidase I).

3. Phylogenetic analyses indicates an intermediate position of South African scallops

The results of three phylogenetic tree construction methods gave the same topology regarding the four main *Pecten* taxa. *P. maximus* appeared as the oldest species, with a sister group composed of the other 3 *Pecten* species. Within this group, *P. sulciostatus* separated first, while *P. fumatus* was the closest relative of *P. novaezelandiae*. This topology supports the BDV model of *Pecten* diversification. However, it is not contradictory with Fleming's model. The reason is that south African scallops could have originated from the ancient *Pecten* stock in the Indian ocean in more recent times than *P. maximus* (Fig. 2).

5. Divergence rates support the BDV model of *Pecten* diversification

The two hypotheses can lead to similar trees, but make different predictions about the magnitude of the differentiation between species. Under Fleming's model, the Atlantic and Australasian clades should have been evolving independently since at least 15 million years, that is the accepted time since the Tethys Sea closure⁶. The BDV model assumes a trans-equatorial migration of the *P. maximus*/*P. sulciostatus* ancestor to South Africa during the Pliocene or early Pleistocene, not earlier than 5 MY ago⁷, and a posterior expansion to Australia and New Zealand in more recent times. Therefore, the genetic divergence between the Atlantic and Australasian taxa should be much smaller under the BDV model than under Fleming's model.

Corrected nucleotide divergence⁹ between pairs of *Pecten* taxa computed from the pooled set of genes studied (Table 1) show very small divergences. This could be due to recent origin or very slow evolutionary rate. In order to discriminate between these two explanations, it is necessary to have an estimate of the evolutionary rate of *Pecten* mitochondrial genes. It is known from fossil data that *P. fumatus* and *P. novaezelandiae* diverged around 1.1 MY b.p.³. Since the genetic divergence observed for this species-pair for the mitochondrial region studied was 0.0060, the evolutionary rate can be established as 2.7×10^{-3} . According with this estimate and genetic distances between *P. maximus* and the remaining species, and assuming no rate heterogeneity within the genus, the diversification of *Pecten* taxa in the southern hemisphere should have taken place 6.4 some MY ago, in good agreement with the BDV hypothesis. The small difference among the distances obtained from the comparison of *P. sulciostatus* with the Australasian species and the distances between *P. maximus* and *P. sulciostatus* also suggest that the diversification of the Australasian species from the South African ancestors took place soon after the colonization of South Africa by the *P. maximus*/*P. sulciostatus* ancestor.

There are other *Pecten* species living in the Indo-West Pacific region that could not be sampled for the present study. *P. modestus* lives in western Australian coasts, and allozyme work suggests a close relatedness with *P. fumatus* and *P. novaezelandiae*. *P. erythraensis* lives in the Red Sea and west Indian ocean¹⁰. *P. afrobedictus* lives in south-east Indian Ocean¹⁰. How these species fit in our present view of *Pecten* diversification remains to be seen.

Fig 1. The five *Pecten* scallop species studied



	PN	PF	PS
PM	0.0309	0.0344	0.0331
PN		0.0060	0.0289
PF			0.0268

Table 1. Corrected distances between pairs of species

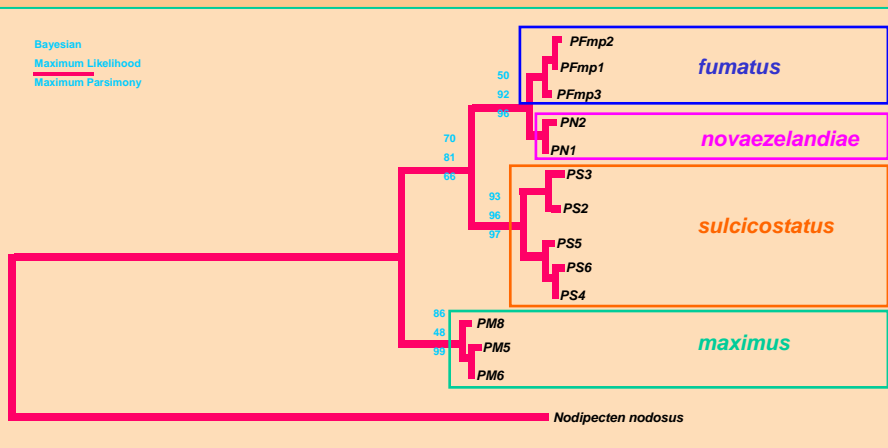


Fig. 5. Phylogenetic tree obtained from the three pooled gene fragments. Numbers in light blue near the nodes indicate posterior probability (Bayesian method) or percent of bootstrap replicates (other methods) supporting the corresponding clades

Materials & Methods

DNA was extracted by a salt precipitation method. PCR was carried out as described, with universal primers (COI, 16S)^{11,12} or newly designed ones (12S)¹³. PCR products were cleaned with MOBIO spin columns and sequenced in an ABI 3700 automated sequencer. Sequences were aligned with CLUSTAL X¹⁴ as implemented in Bioedit¹⁵. Phylogenetic analyses included maximum parsimony (MP), maximum likelihood (ML) and Bayesian tree construction. MP was carried out with PAUP¹⁶ by TBR. ML was carried with PAUP¹⁶ under a Tamura-Nei+G model, which was selected with MODELTEST¹⁷. Bayesian analysis was performed with MrBayes v3.1¹⁸. Confidence bootstrap values for MP and ML were obtained with PAUP, and are based on 1000 and 200 replicates, respectively. Divergence between species, corrected for intraspecific diversity, was estimated under a Kimura Two-Parameter model with MEGA¹⁹.

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