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**NATIONAL STANDARD OF THE
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Zhikong scallop

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1 Scope

The document fixes the scientific name and classification, the main morphological and structural characters, the growth and reproductive characteristics and the cytogenetic and molecular genetic characteristics of the Zhikong scallop, *Chlamys farreri* (Jones & Preston, 1904), describes the corresponding test methods and lays down the rules of judgement.

It applies to the germplasm testing and identification of the Zhikong scallop. The terms and definitions of GB/T 22213 apply, and the other normative references are GB/T 18654.6,

GB/T 18654.12 and GB/T 32757.

4 Scientific name and classification

The scientific name is *Chlamys farreri* (Jones & Preston, 1904).

The taxonomic position given is the phylum Mollusca, the class Lamellibranchia, the order Pterioidea, the family Pectinidae and the genus *Chlamys*.

5 Main morphological and structural characters

The shell is bivalve and fan-shaped, the two valves almost equal in size, the right one slightly flatter and the left one slightly convex, with a nearly round ventral margin. The umbo lies on the dorsal margin and is slightly convex; from it a larger anterior ear projects forward and a smaller posterior ear backward, both triangular, the two ears differing in shape. The ventral face of the anterior ear of the right valve is hollowed backward, forming the comb-like opening through which the closed shell communicates with the outside, and the upper edge of the right valve at the ventral side of that opening bears small comb-like teeth. The outer surface is mostly purple-brown or light brown, with yellow-brown, apricot-red or greyish white among it, and the inner surface is white, brown or pink. The number and shape of the radial ribs differ between the two valves. Figure 1 shows the external form and labels shell height, shell length, shell width, anterior ear length and posterior ear length.

The countable characters are 6 to 10 comb-like teeth, and radial ribs that differ between the valves, the left valve carrying about 10 coarse main ribs and the right valve about 20 coarse ribs.

The measurable characters are a shell height slightly greater than the shell length, an anterior ear about twice as long as the posterior ear, and an umbo angle of about 60 degrees.

Internally the mantle margin has no fusion points and bears well-developed tentacles of unequal length, with many mantle eyes at their bases. The foot is short and rod-shaped with a byssal groove on its membrane face, and the byssus is well developed and fine and thread-like. Figure 2 labels the stomach, ventricle, auricle, rectum, pedal retractor muscle, adductor muscle, anus, mantle eye, mantle tentacle, gill, kidney, intestine, foot, liver, labial palp and mouth.

6 Growth and reproductive characteristics

Table 1 gives the shell height in millimetres of Zhikong scallops of different age groups from natural sea areas: 22.8 to 25.0 for age 0 plus; 49.5 to 72.0 for age 1 plus; 64.2 to 81.0 for age 2 plus; 70.3 to 86.0 for age 3 plus; and 76.1 to 88.0 for age 4 plus. A footnote states that the figures are measurements taken at the end of the year in question.

Sexual maturity is reached at one year of age in natural sea areas. There are two breeding seasons, the first peak falling in May to July and the second in September to October, and the water temperature suited to breeding is 16 °C to 23 °C.

Spawning takes place several times a year. A female with a shell height of 6 cm to 7 cm carries from 8 million to 10 million eggs and releases about 2 million at one spawning. The eggs are demersal, with a diameter of 69 µm to 72 µm.

7 Cytogenetic and molecular genetic characteristics

The somatic chromosome number is $2n = 38$.

A reference sequence of 592 base pairs of the 16S ribosomal RNA gene fragment is reproduced in full in the document.

The intraspecific genetic distance calculated by the Kimura two-parameter model shall be less than 2 %.

9 Test methods

The external morphology is observed by eye in natural light; the measurable characters are measured in natural light with a vernier caliper and a protractor, as shown in Figure 1; the internal structure is observed under a dissecting microscope after dissection.

Age is judged from the annual rings on the shell, in natural light and after the shell has been cleaned, against Figure 3, which labels the rings of the first to the fifth year.

The number of eggs carried is measured in accordance with GB/T 18654.6. At least 50 scallops with well-developed gonads are taken at random; after spawning the water in the tank is stirred so that the eggs are evenly distributed, samples are taken with a long glass tube at the four corners and at the centre and mixed, the eggs in each millilitre of water are counted on a haemocytometer, the count is repeated three times and the arithmetic mean is taken, and the total number of eggs is calculated from the total volume of water. The number of scallops that spawned is counted by dissection, the number of eggs per scallop is calculated, and the count is repeated three times and averaged. The egg diameter is measured under a microscope with an eyepiece micrometer on at least 30 eggs, and the arithmetic mean is taken.

For the chromosome preparation the gill tissue of the scallop is used, with a colchicine concentration of 0.005 % to 0.010 % and a treatment time of 15 min to 40 min, hypotonic treatment in 0.075 mol/L potassium chloride for 10 min to 30 min, fixing in Carnoy fixative for 15 min to 20 min, dissociation in 50 % acetic acid for 1 min to 5 min, and dropping of the cell suspension, frozen or hot, to make the preparation, which is stained with Giemsa for 10 min to 15 min and examined under the microscope; the rest follows GB/T 32757.

Chromosomes are counted by the method of GB/T 18654.12. The molecular genetic

characteristics are determined by the method of Annex A.

10 Rules of judgement

Where the test result meets the requirements of Clause 5, the sample is judged to be of the species.

Where one of the following applies, the content of further clauses is to be tested as well and the species judged from the results taken together: where Clause 5 cannot be tested or cannot be judged with certainty, the content of Clause 7 or of Clause 8 is tested in addition; where a third party asks for all or part of the content of Clause 6 to be tested; and where all items are tested.

A Annex A (normative) Molecular genetic characteristics

For the total DNA extraction about 100 mg of muscle tissue is taken, cut up and digested with proteinase K, and the total DNA is extracted by the standard phenol-chloroform method or with a kit.

The primer sequences given are 16S AR and 16S BR, each written out in the document from the five prime to the three prime end.

The reaction mixture is made up of 2.0 mmol/L magnesium chloride, 0.2 mmol/L deoxynucleoside triphosphates, 0.2 μ mol/L primers, 1 mL of DNA template, 1 unit of Taq polymerase and 2.5 μ L of 10 times buffer, with sterile water added to a volume of 25 μ L. The volume of template is given as 1 mL in a reaction of 25 μ L in the original text and has been rendered as it stands.

The amplification programme is predenaturation at 94 °C for 2 min; then 35 cycles of denaturation at 94 °C for 45 s, annealing at 50 °C for 1 min and extension at 72 °C for 1 min; and a final extension at 72 °C for 5 min. A blank control without DNA template is set up in every run and the products are checked by electrophoresis on a 1.4 % agarose gel. After electrophoresis the products are recovered, purified and sequenced in both directions.

The genetic distance between the sequence of the sample and the reference sequence is calculated with the Kimura two-parameter model.