ANGLERFISH AGEING GUIDE

by

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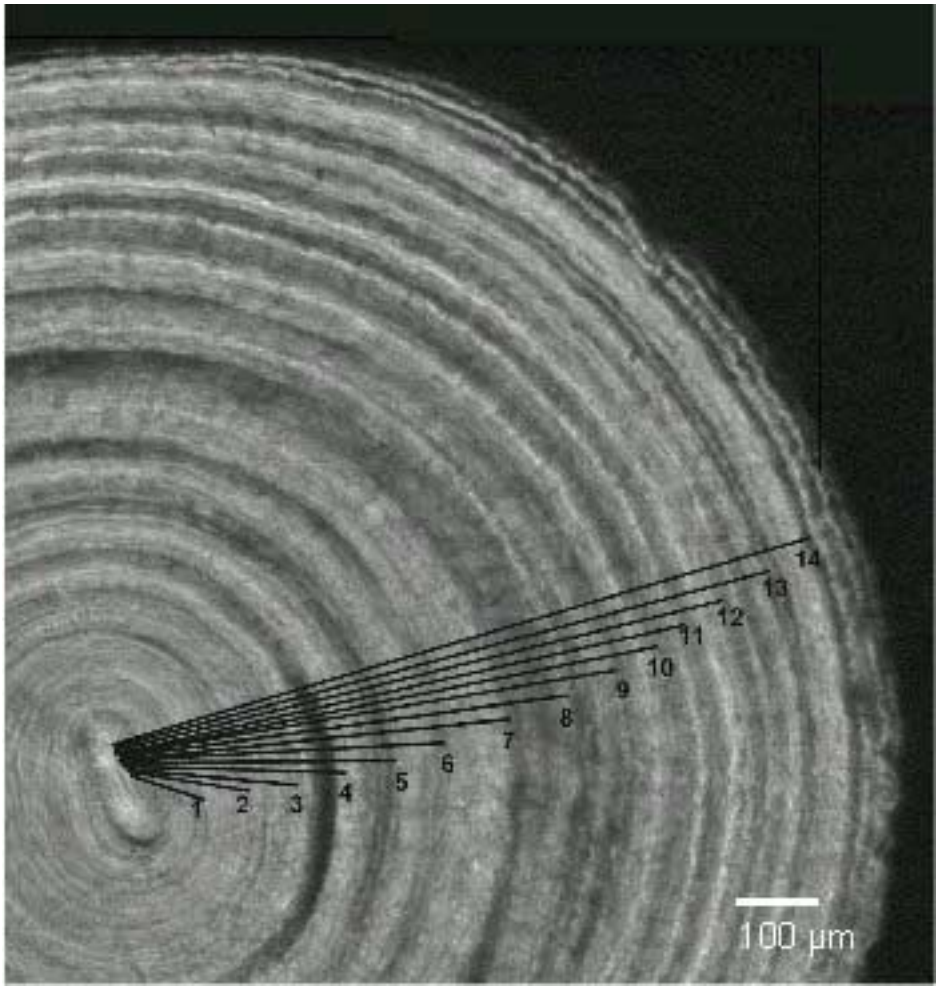

Summary

The objective of the present ageing guide is to make a compilation of all the information necessary to age the two species of European anglerfish (*Lophius piscatorius* and *L. budegassa*). The used calcified structure is the first dorsal fin ray (*illicium*). Anglerfish ageing is generally recognised as a difficult task. Hervé Dupouy, from IFREMER, Lorient (France), started to implement in the eighties a routine ageing procedure, based on *illicia* transversal sections. After recognising the benefits of this procedure and the clearer annual ring identification, compared to otoliths, researchers from Spain and Portugal followed Hervé's work, in order to provide annual data for stock assessment. Since the beginning of the nineties, four *illicia* ageing workshops were held in order to improve methodologies and uniformity in the ageing criteria. This ageing guide results from the work developed during these workshops and the objective is to present all the necessary information to age anglerfish. This way, the introduction in section 1 makes a summary of the main biological particularities of these species, section 2 contains all the methodology to obtain *illicia* transversal sections, section 3 describes the ageing criteria and in section 4 are *illicia* images with marked annual rings.

Participants

(By alphabetical order of last name)

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Contents

1. INTRODUCTION

Black anglerfish (*Lophius budegassa* Spinola, 1807) and white anglerfish (*L. piscatorius* Linnaeus, 1758) are important species in European fisheries. They are very similar, being distinguished by the colour of the peritoneum (*L. budegassa* black and *L. piscatorius* white) or by the number of rays in the second dorsal fin (*L. budegassa* 9-10 and *L. piscatorius* 11-12) (Caruso, 1986). Both black and white anglerfish are typical bottom living species, the former having a depth range between 70 m and 800 m and the latter extending to depths >1000 m (Dardignac, 1988; Azevedo and Pereda, 1994). Black anglerfish has a more southern distribution (Mediterranean and Eastern North Atlantic from British Isles to Senegal) compared to white anglerfish (Mediterranean, Black Sea and Eastern North Atlantic from Barents Sea to the Straits of Gibraltar), but there is considerable overlap (Fig. 1).

The spawning season of *L. piscatorius* is poorly described in the literature, but is generally from late winter to summer. Afonso-Dias and Hislop (1996) observed spawning females from November to May in Scottish waters, Quincoces *et al*. (1998a) described the spawning period between May and August in the Cantabrian Sea. In Norwegian waters it has been observed between June and August (Staalasen, 1995). The poor description of the spawning period is due to the difficulty in

obtaining mature females from the fishery and research surveys. The spawning period is described better for *L. budegassa*. Azevedo (1996) observed spawning during October to March off the Iberian coast. Quincoces et al. (1998b) observed it between May and July in the Cantabrian Sea and Duarte et al. (2001) between November and February off the Iberian coast.

The maturation process of male and female gonads is well described in the literature (Afonso-Dias and Hislop, 1996 and Quincoces *et al*., 1998a,b). The length at first maturity seems to be quite long. Females of *L. piscatorius* mature at around 70-75 cm while males reach maturity at a shorter length (50 cm). For *L. budegassa*, females reach first maturity from 50 to 65 cm and males reach it between 35 and 40 cm.

The reproduction of the *Lophius* genera is very unique compared to other teleost species, the ovaries are ribbon like and the eggs are released in floating gelatinous matrixes (Armstrong et al., 1992; Afonso-Dias and Hislop, 1996 and Quincoces *et al*., 1998a,b). This unique characteristic makes it difficult to observe the species during routine larval-egg surveys.

The growth of *L. piscatorius* and *L. budegassa* has been studied based on Sagitae Otoliths (Crozier, 1989 and Tsimenidis, 1984) and *Illicia* (Dupouy *et al*., 1986; Duarte *et al*., 1997; Landa and Pereda, 1997 and Landa *et al*., 2001). Studies comparing the two structures showed that with *illicia* higher agreement between readers were achieved (Dupouy pers. com.; IFREMER, 1991, Staalasen, 1995). Observations have shown that *L. piscatorius* attains higher lengths than *L. budegassa*. In both species, females achieve greater lengths. For *L. piscatorius* females reach an L_∞ between 160 and 170 cm compared to males with an L_∞ between 110 and 130 cm (Dupouy *et al*., 1986 and Landa *et al*., 2001) and *L. budegassa* females reach an L_∞ between 90 and 110 cm compared to males with an L_∞ between 70 and 85 cm (Dupouy *et al*., 1986; Duarte *et al*., 1997 and Landa *et al*., 2001).

For age determination it is important to understand the early life cycle of the species. After spawning of *L. piscatorius*, there are indications of yolk sac duration of between 15 and 20 days and that the pelagic phase is around 3 to 4 months duration. Studies based on otolith daily increments shows that pelagic specimens between 7 and 11 cm

are between 80 and 120 days (Hislop *et al*., 2001). Settlement probably occurs when specimens reach around 6 cm or greater (Russel, 1976 and Hislop *et al*., 2001). There is no data in the literature for the early life cycle of *L. budegassa* but it is assumed to be similar.

The early life cycle of the two species has a marked influence on the microstructure of the *illicia*. Observing the *illicium* under sweeping electronic microscopy (SEM), the area of its nucleus is distinguished (Fig. 2). This area, when being attacked by EDTA used for the preparation of the samples for its vision under SEM, presented multiple holes, possibly due to the heterogeneous crystallization of this area of the *illicium* (Quincoces, 2002). It shows an amorphous crystallization and in an axis different to that given in the rest of the *illicium*. This nucleus also is similar in length and in shape to the nucleus observed under photonic microscopy. It is considered to be a consequence of a change in the life cycle (changing from planktonic to benthic living), and is therefore designated as the benthic ring.

Age readings based on *illicia* have been applied for stock assessment of both species. In spite of recent ageing studies, there are still some doubts concerning age validation and ageing precision between readers. In order to improve agreement between readers and this way provide more precise data for stock assessment, four workshops were carried out with different objectives and in different situations concerning biological knowledge of the growth of both species. The first ageing Workshop for Anglerfish was carried out in 1991 (IFREMER, 1991) involving readers from France and Spain. The main objective was to define clearly an ageing structure to perform ageing for stock assessment purposes. Best results were obtained with the *illicium*, which was adopted. The second workshop held in 1997 (IFREMER, 1997) was conducted with additional participation by Portugal and the main objective was to increase ageing precision between readers. The third workshop was carried out in 1999 (Anon., 1999) with additional participation by Ireland and the main objective was to establish and describe ageing criteria in order to increase the ageing precision between experienced readers. The fourth workshop was held in 2002, after an *illicia* exchange that included digital images. The main objective was to discuss the exchange results and to analyse the digital images, where each reader marked the annual rings. The images were considered a very important tool to discuss ageing criteria.

2. MATERIALS AND METHODS

The interpretation of growth structures in *illicia* is widely accepted as one of the most reliable methods for ageing anglerfish. For this method it is necessary to prepare a section 0.5 cm from the base of the *illicia* with a thickness of 0.5 mm or less in which the age readings are performed using a microscope.

2.1. *Illicia* mounting and sectioning

The *illicia* mounting has the objective of including the *illicia* in resin that serves as a support for the sectioning. The material necessary is a mould, a set of chemical compounds and a cutting machine. The practical procedure for the *illicia* mounting and sectioning is also described.

2.1.1. The mould

The mould is basically a base plate with two longitudinal strips and three lateral strips. These five strips are screwed to the base as shown in Fig. 3. The base plate and the strips of the mould are composed of aluminium and the screws are made of stainless steel.

2.1.2. Materials

To prepare the blocks of black resin the following material is necessary (Fig. 4):

- Honey Wax (Mold Release Compound)
- Resin (SP106 / Multi-purpose Epoxy System)
- Slow Hardener (SP106 / Multi-purpose Epoxy System)
- Epoxi Pigment Black (The proportions used are 81% resin for 16% slow hardener for 3% epoxi pigment black)
- glass microscope slides
- glue (entellan)

2.1.3. Slicing machine

The slicing machine (Fig. 5) can be of different types. Nevertheless it is essential to have the following characteristics:

- Cutting speed of 2000 rpm or higher,
- Diamond sectioning blade,
- Cooling system

2.1.4. Preparation of the base and mounting the *illicia*

The inner surfaces of the mould are lightly smeared with a releasing agent (Honey Wax) and a first layer of resin is poured in the mould. The mould is then placed on a level surface (Fig. 6) and when the resin becomes viscous / sticky the mounting of the *illicia* can start.

A strip of "spaghetti" is placed at the extreme left-hand side of the mould. The *illicia* are placed in the mould in straight and parallel lines. The rows start at the top left-hand corner and work from left to right (Fig. 7). The "spaghetti" marks the left side of the slices and this way it is possible to identify each *illicium*. All this process can be made manually or using a specific hardware (Fig. 8).

When all the rows have been filled with *illicia*, and the resin is less viscous (to avoid the *illicia* moving), a further layer of liquid black resin is poured on top, filling the mould, which is placed again on a level surface.

2.1.5. Cutting the *illicia* sections

Each polyester block is set up in the machine and cut separately. A simple jig, permanently fixed to the table of the machine is used to hold the block under the cutting disc. The row of *illicia* is positioned under the cutting disc (Fig. 9) and the cutting process begins (following the specification of the different types of cutting-machine) resulting in sections of 0.5 mm or less in thickness.

2.1.6. Mounting the slices

Finally the sections are fixed permanently in glass microscope slides. This is done using glue, which fix them to labelled microscope slides (Fig. 10).

2.2. Observation

Microscopes / Software

An image analysis system is used, composed of a microscope (Zeiss) with a video camera (Sony Model DXC-930P / 3CCP Colour Video Camera CCD-IRIS). The magnification is 100X and transmitted light is used. This system allows the treatment of the images (mark the rings and measure distances) (Fig. 11).

3. AGEING CRITERIA

Anglerfish ageing using *illicia* consists of identifying dark and light rings (Fig. 12, Fig. 13). For age determination only the dark rings are counted. For this we assume that one dark ring represents one year growth. At times these rings are well defined and clearly visible, but most of the time, rings appear doubled and are not well defined, which makes ring identification very difficult. From the open discussion and communal interpretation of *illicia* sections during the 4th International Ageing Workshop on European Anglerfish, some peculiarities inherent to *illicia* ageing were defined:

- It is important to play with and adjust the light and focus of the microscope, to identify the pairs of dark and light rings, and to try to find a general pattern of growth. Unlike otoliths, where ring widths tend to decrease as you approach the edge, in *illicia,* rings remain a similar width apart throughout the section. Rings close to the edge may even be wider apart than those closer to the nucleus (Fig. 12, Fig. 13).

- Rings in *illicia* differ in composition. As a result, the surface appears rippled, alternating between high and low ridges. The differences in these levels relate directly to the dark and light rings. This characteristic is very apparent from research carried out using scanning electron microscopy (Fig. 14).

- Rings may not be visible in all the axes of the section. Defined rings, which are clearly visible in one part of a section may be less defined or even appear to double in another part of the section. The counting should be based upon the area where good contrast between rings exists.

- The next step is to identify the position of the first annual ring, and to confirm this by measuring its diameter. For this reason it is necessary to know the following.

    - The first well-marked ring usually observed is considered to be a consequence of a change in the life cycle (changing from planktonic to benthic living), and is therefore designated as the benthic ring (Fig 15, Fig. 16). The next ring is considered to be the first annual ring. When identifying the first annual ring, the diameter of the benthic ring can be of assistance. The distance of the first annual ring from the benthic ring is usually not greater than half the distance of the diameter of the benthic ring.

    - In *L. piscatorius*, the first ring tends to be oblong in shape and the mean horizontal diameter of the first ring tends to be between 200 and 300 μm (Fig. 15). For *L. budegassa*, the first ring tends to be circular in shape and the mean diameter of the first ring tends to be at 80 μm (between 60 and 100 μm) (Fig. 16).

- To identify the outer ring it is very important to look at the edge of the *illicium*. For this it is essential to know the quarter (or month) in which the sample was taken. This will determine whether or not the ring at the edge is to be counted in the age reading. At times the outer ring(s) are not visible in the whole *illicium*, this may be because the section has not been cut perpendicularly (Fig. 17). When a dark ring appears at the edge in Q1, it should be counted and included in the age reading. If a similar ring appears in Q4 it should not be counted or included in the age reading.

- It is recommended to read *illicia* of similar length group fish together, and also to begin with the clearest *illicia* sections. This is a good exercise to help train the eye in identifying the typical pattern of *illicia*. Because the first rings in younger fish are often difficult to define, it is easier to begin reading the *illicia* from the middle of the fish length range to establish the growth pattern of these first rings. For example in *L. piscatorius* this corresponds with lengths in the range of 60 to 90cm, and in *L. budegassa* it is between 50 to 70cm.

- The length of the fish can be a useful piece of information in ageing *illicia.* It is recommended to do a first reading and afterwards to check that the age reading lies within the possible mean fish length range at that age. For example for *L. piscatorius* a fish of 20cm will be aged between 1 - 3 years. For *L. budegassa* the same fish would be expected to be between 2 - 3 years.

- The ageing of this species is not easy. As an example we can see that within this *illicia* exchange, even with samples that were originally considered to be clear, the expert readers assigned the following credibility percentages. 55% were considered to be of medium credibility, and only 30 % were considered to be high, a further 15 % were found to be bad. It was also found that 2% of the *illicia* were unreadable.

- Confusion after some ages (age 6) may be related to first maturation or any other unidentified life – history event, which causes changes in the growth pattern.

4. REFERENCE COLLECTION OF *ILLICIA* IMAGES OF AGREED AGES

The following reference collections of illicia images of agreed ages were prepared.

4.1. *L. piscatorius*

Image number	Agreed age	File name	Collection area	Comments
1	1	14 3b-1 1999	South	Total Agreement by experienced readers
2	2	289-97-5b-4	North	Total Agreement by experienced readers
3	3	67-99-5b-3	North	Total Agreement by experienced readers
4	3	2-1b-4 2000	South	Total Agreement by experienced readers
5	4	60-99-3a-3	North	Total Agreement by experienced readers
6	5	73-99-1b-4	North	Total Agreement, better image
7	5	13-4a-21999	South	Total Agreement by experienced readers
8	6	2b-5	North	Modal Age agreed, but different rings selected
9	8	66-99-4a-7	North	Disagreement on 3rd and 4th rings
10	9	11-00-1a-1	North	Good agreement by R5 &R6, R3= N-1
11	10	84-99-2A-3	North	Total Agreement by experienced readers
12	11	15-4b-5 1999	South	R5&3=11, R6=12, however chose very similar rings
13	12	114-99-2a-1	North	Disagreement on one ring

14	12	8-4b-3-2000	South	R3 differs on ring 1 and 11
15	13	238-97-1a-4	North	R1,5 &6 =13, similar rings, R3=11
16	14	100-99-3b-3	North	R5&6 in total agreement, R3=11
17	16	77-99-5b-8	North	Total Agreement by experienced readers
18	24	9-1b-3 1999	South	Age 24-29, image looks good, different rings chosen
19	25	9-1b-2 1999	South	Total Agreement by experienced readers

4.2. *L. budegassa*

Image number	Agreed age	File name	Collection area	Comments
20	1	A-2	South	Total Agreement by experienced readers
21	2	A-7	South	Total Agreement by experienced readers
22	3	B-3	South	Total Agreement by experienced readers
23	4	19-1998-5b-8	North	Total Agreement by experienced readers
24	5	C-7	South	Total Agreement by experienced readers
25	6	3-1999-3b-2	North	Total Agreement by experienced readers
26	7	39-1998-3a-3	North	Total Agreement by experienced readers
27	8	G-7	South	R3 missed 3rd ring
28	9	6-1999-3b-4	North	R5&R6=9, similar rings, R3=6
29	10	237-1997-5a-5	North	Total Agreement by experienced readers
30	11	92-1999-5b-10	North	Agreement on all rings except 1st by R3
31	12	K-6	South	R5&6 = 12, R3=8, Good illustration of applying different ageing criteria.
32	15	N-1	South	R5&6=15, R3=13
33	19	N-5	South	R3=17, R6=19 and R5=20, R5&6 chose similar rings
34	21	O-3	South	R3=16, R5&R6=21, but chose some different rings

Figure 1. Distribution area of *L. piscatorius* and *L. budegassa*

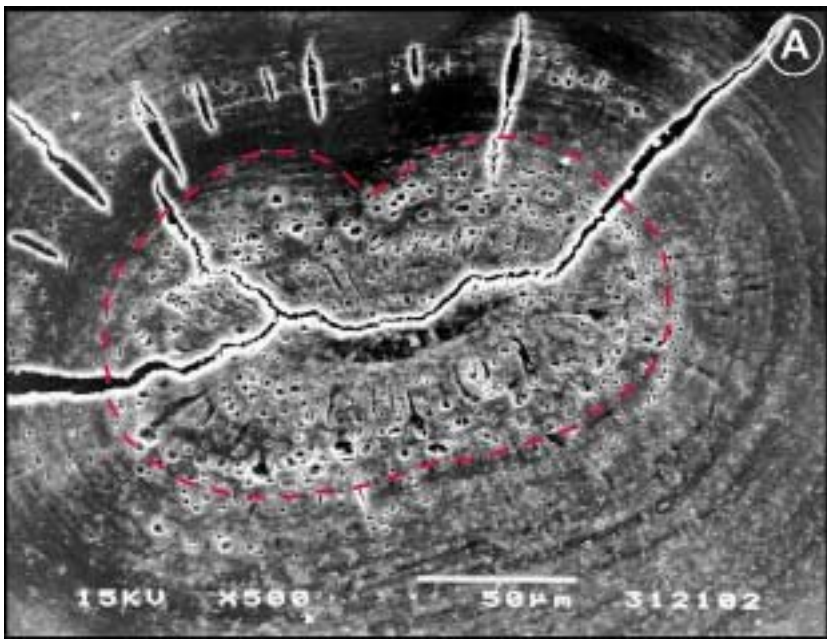

Figure 2. Microphotograph with sweeping electronic microscopy at 500X of the central area of an *illicium* of *L. piscatorius* of 63 cm and 8 years of age. The nucleus of the *illicium* is marked with a line discontinuous.

Figure 3. The mould.

Figure 4. Materials: Resin, pigment, wax and entellan.

Figure 5. Slicing machine.

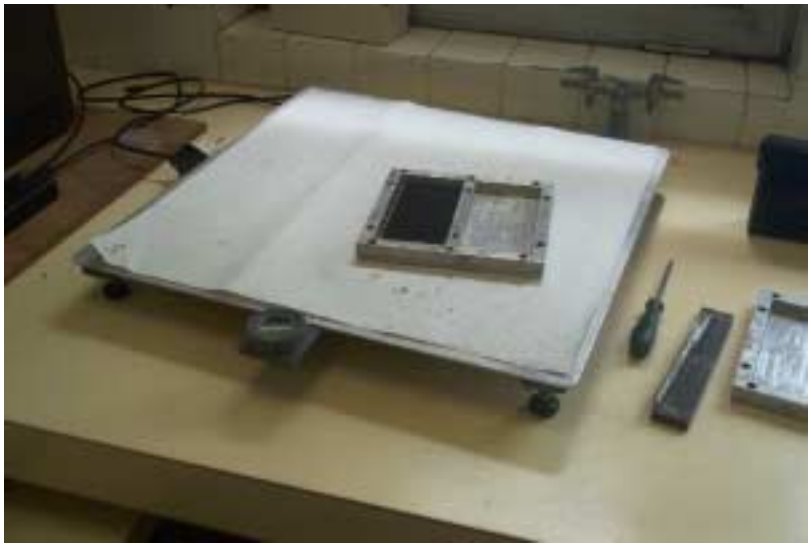

Figure 6. Level surface with mould.

Figure 7. Mould with a “spaghetti” on the left side. *Illicia* are placed from left to right.

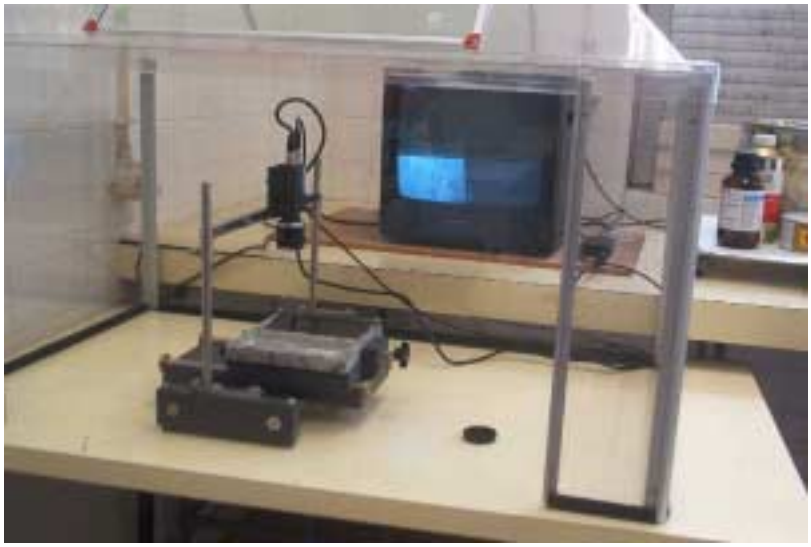

Figure 8. Mounting system.

Figure 9. Resin block positioned in the slicing machine, ready to begin the slicing process.

Figure 10. Microscope slide with two *illicia* sections.

Figure 11. Image analysis system.

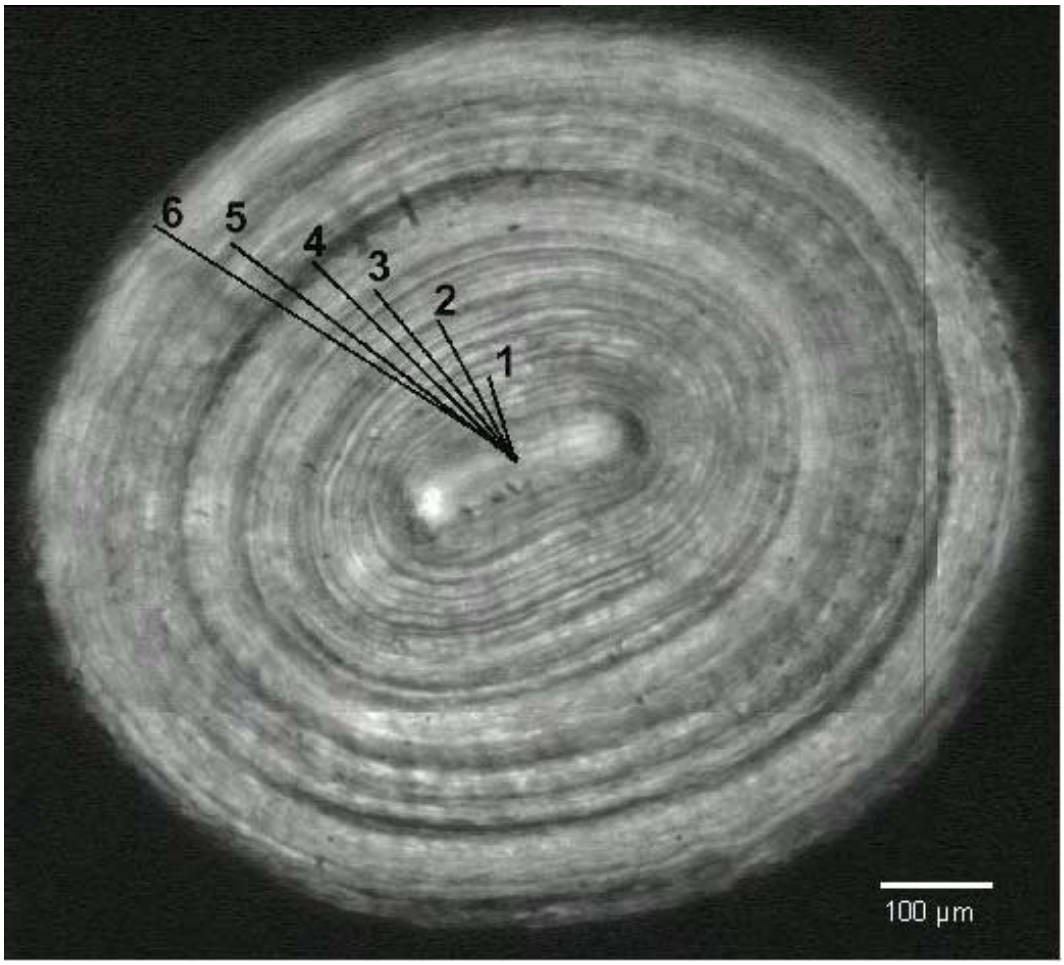

Figure 12. *Lophius piscatorius* with 51 cm in length. There are 6 annual rings visible. Distances between the majority of the rings are equivalent but rings 5 and 6 are more separated compared to 3 and 4 or 2 and 3.

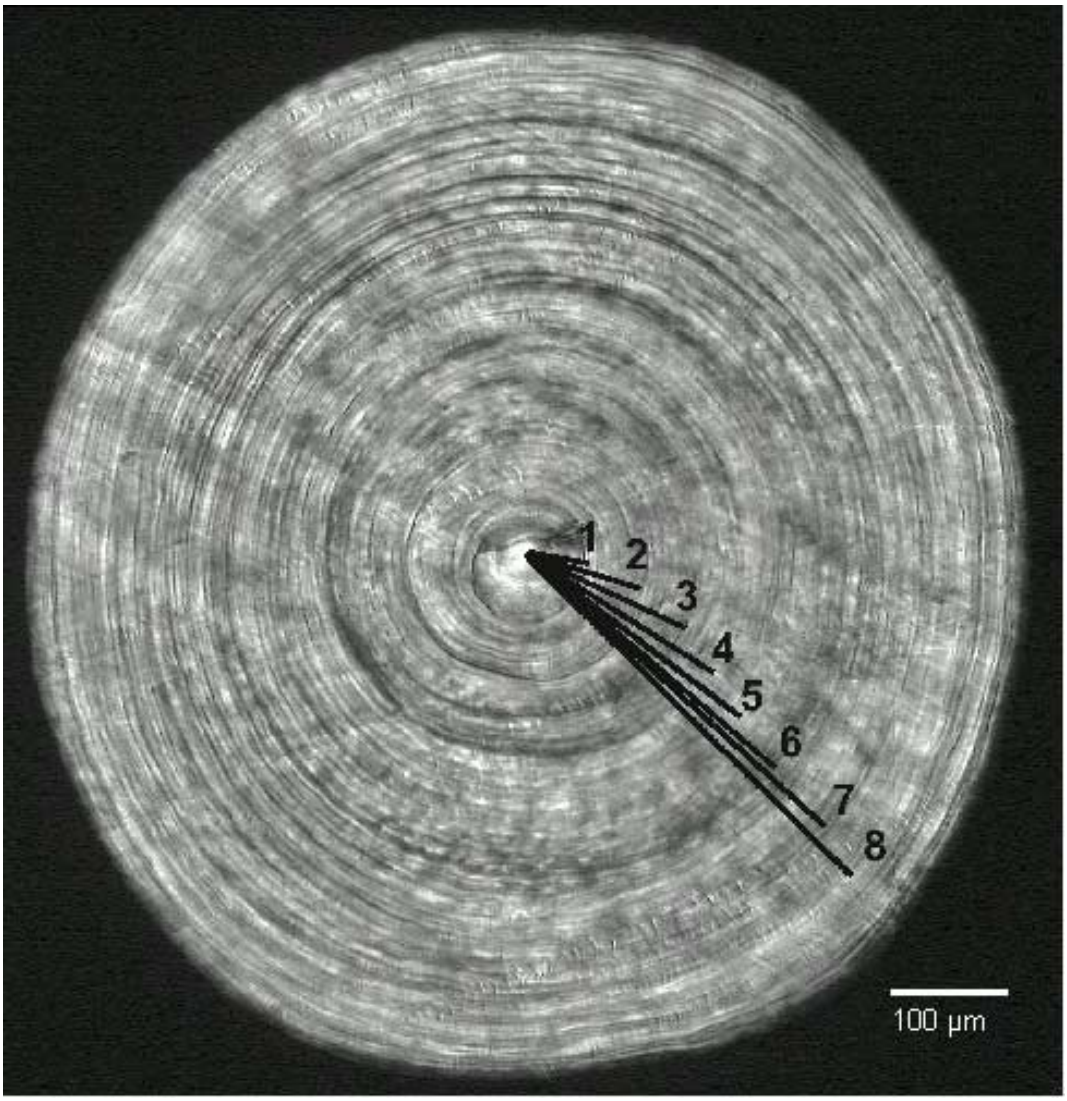

Figure 13. *Lophius budegassa* with 52.7 cm in length. There are 8 annual rings visible. Distance between rings 7 and 8 is greater compared to distance between rings 6 and 7 or 4 and 5.

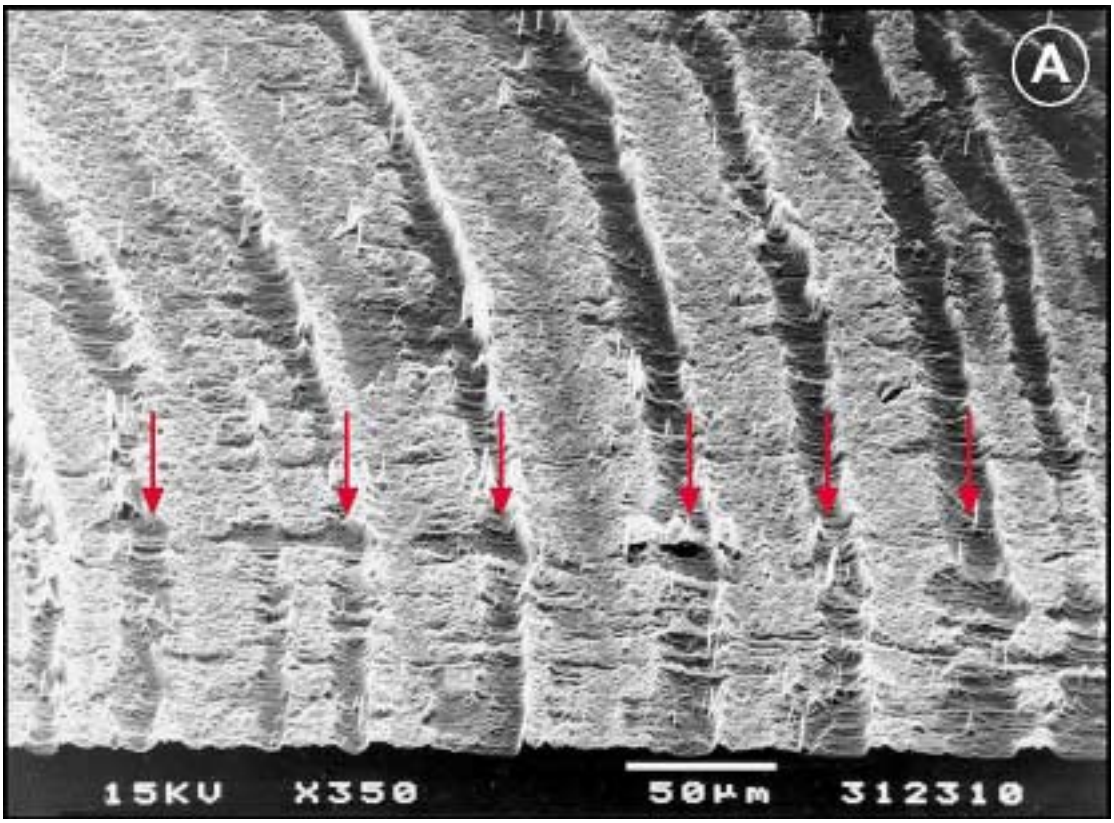

Figure 14. *Illicium* image obtained using scanning electron microscopy. The surface appears rippled, alternating between high and low ridges (dark and light rings).

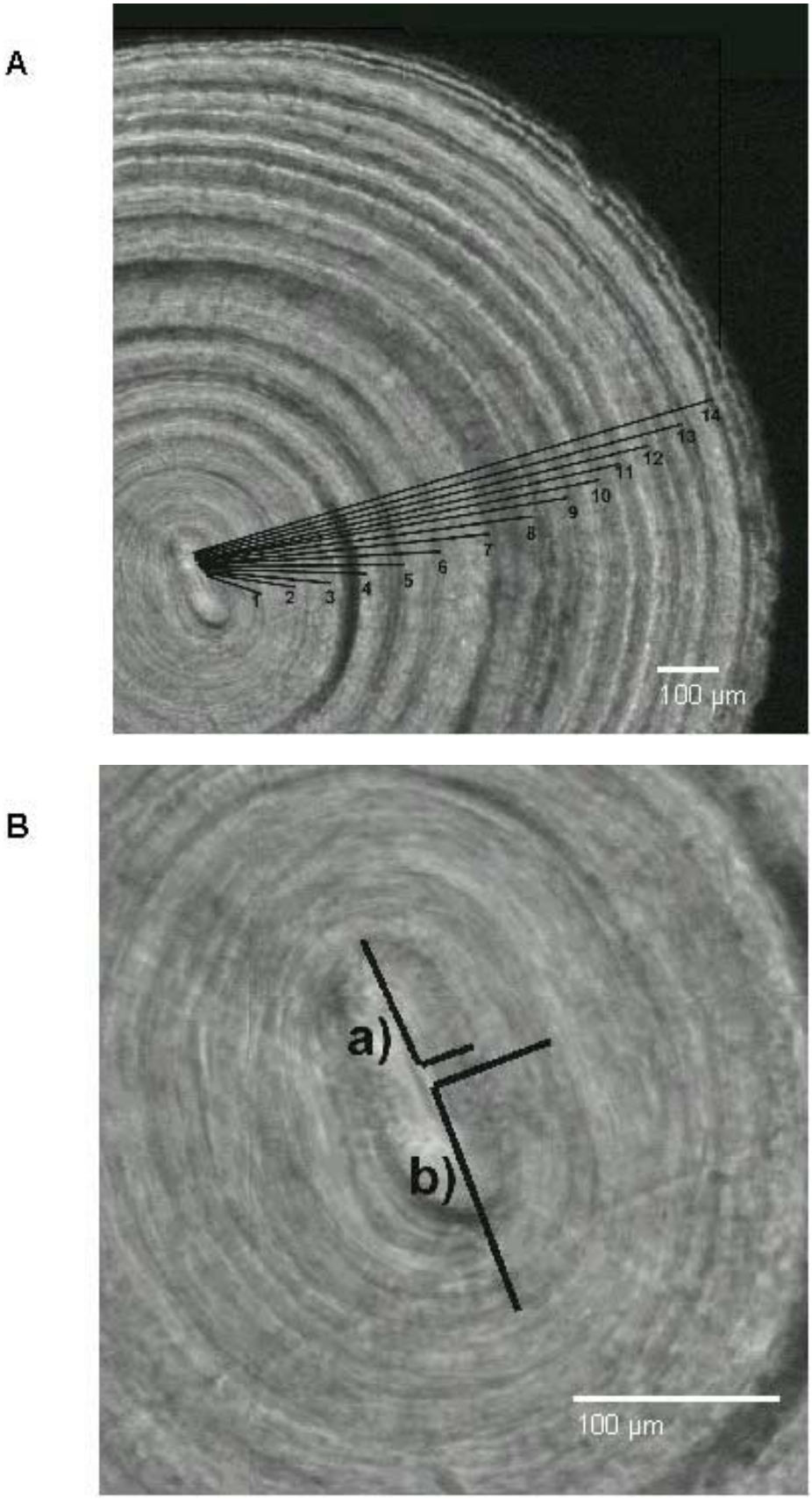

Figure15. *Lophius piscatorius* with 91 cm in length. In Image **A** are the identified annual rings (14 annual rings) and Image **B** contains for the same *illicium*, only the central part. Image **B** shows the oval shape of the first rings characteristic of this species and the benthic ring is marked as a) and the first annual ring is marked as b).

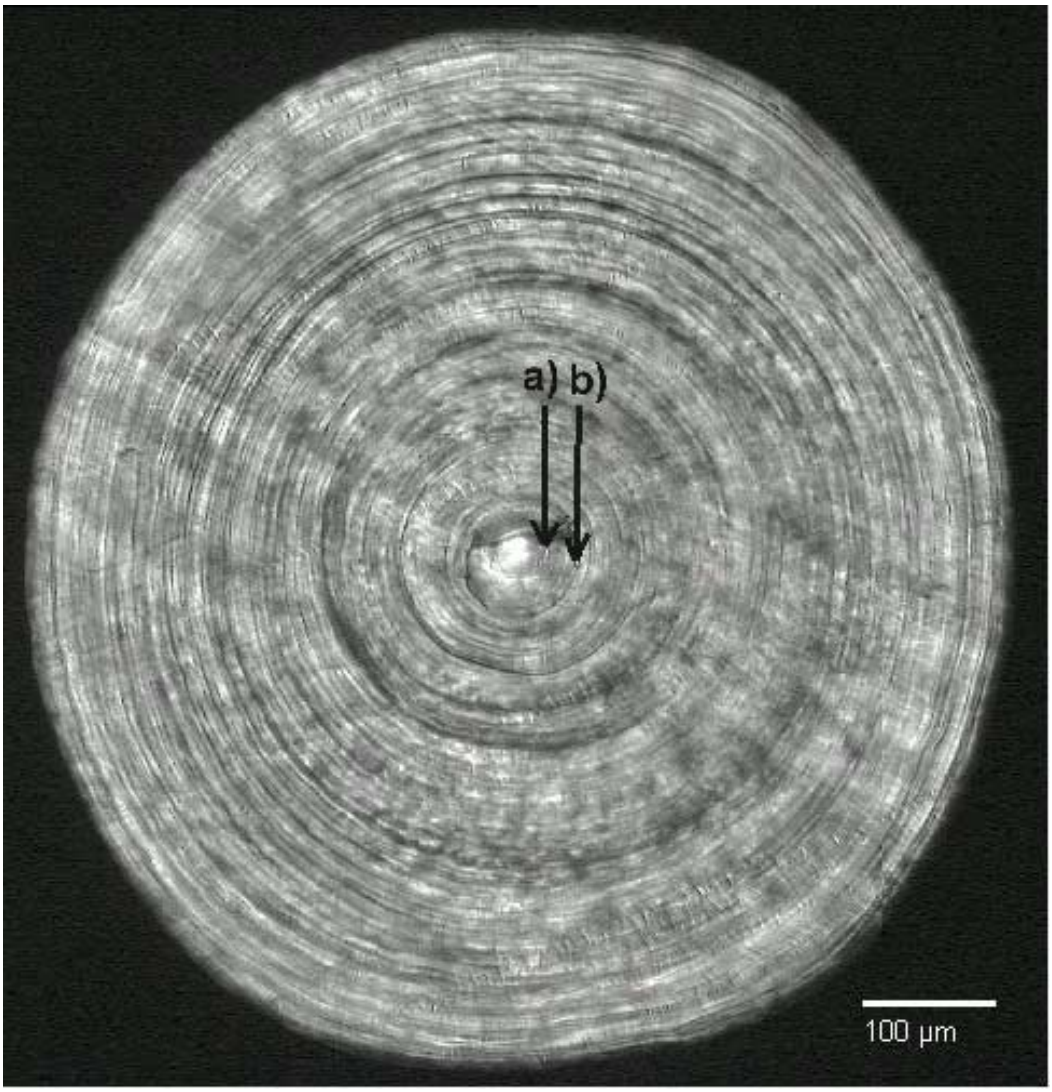

Figure 16. *Lophius budegassa* with 47.9 cm in length. Benthic ring is marked as a) and first annual ring is marked as b). Annual rings and nucleus tend to be circular in shape, what is a characteristic of this species.

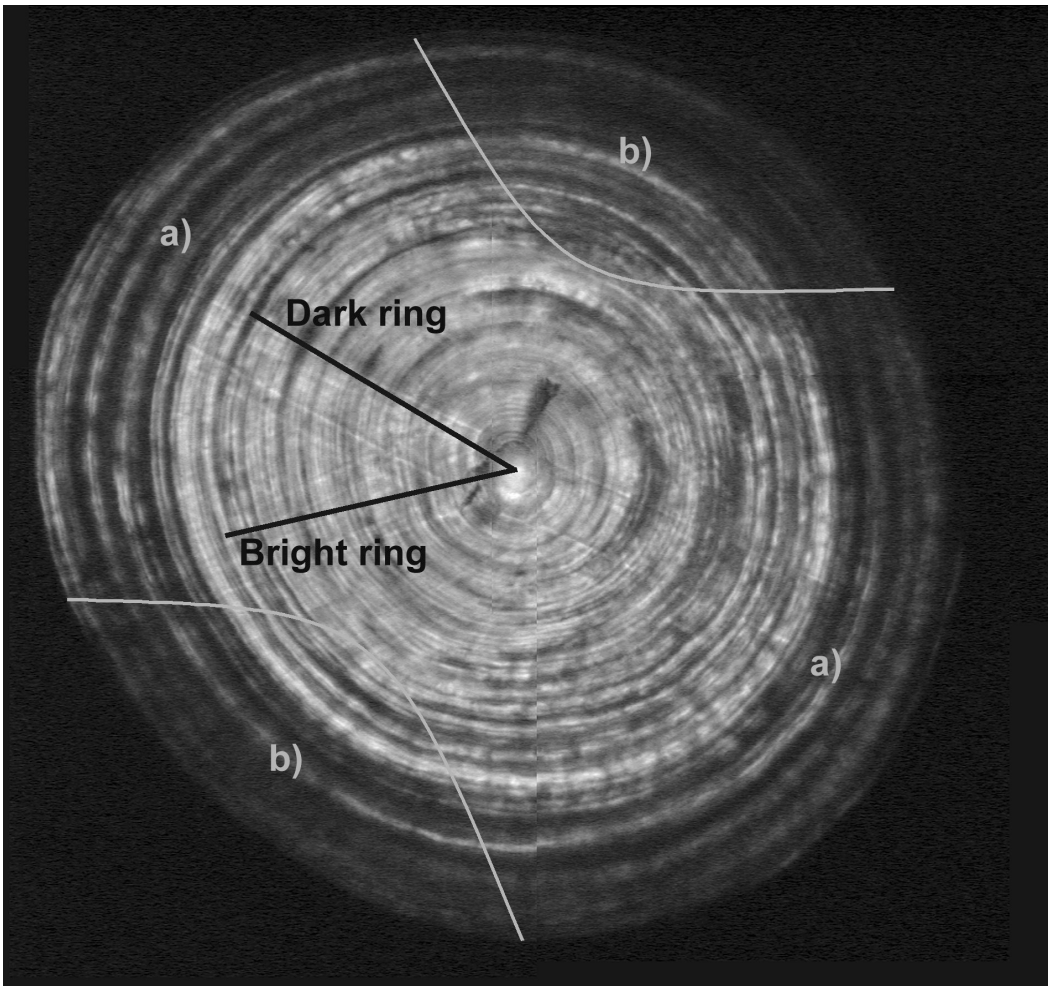

Figure 17. *Lophius budegassa* with 64 cm in length. Region a) of the *illicium* with good contrast between rings and region b) with low contrast. Some rings visible in region a) are not distinguishable in region b). Relative to the ring coloration, there are two well marked dark rings in a certain region of the *illicium* and in another region of the cut the dark part disappears and a bright part is very visible and easy to count.

Image 1. *L. piscatorius.* Agreed Age: 1. File name: 14 3b-1 1999. Collection South. Total Agreement by experienced readers.

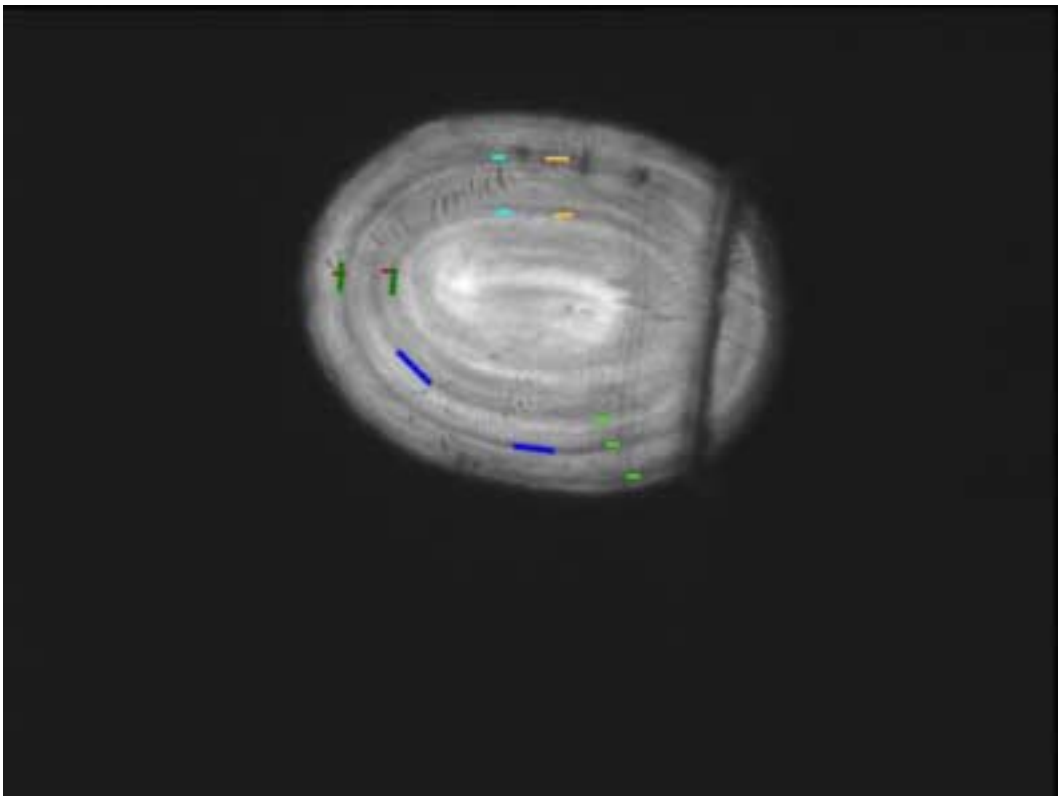

Image 2. *L. piscatorius.* Agreed Age: 2. File name: 289-97-5b-4. Collection North. Total Agreement by experienced readers.

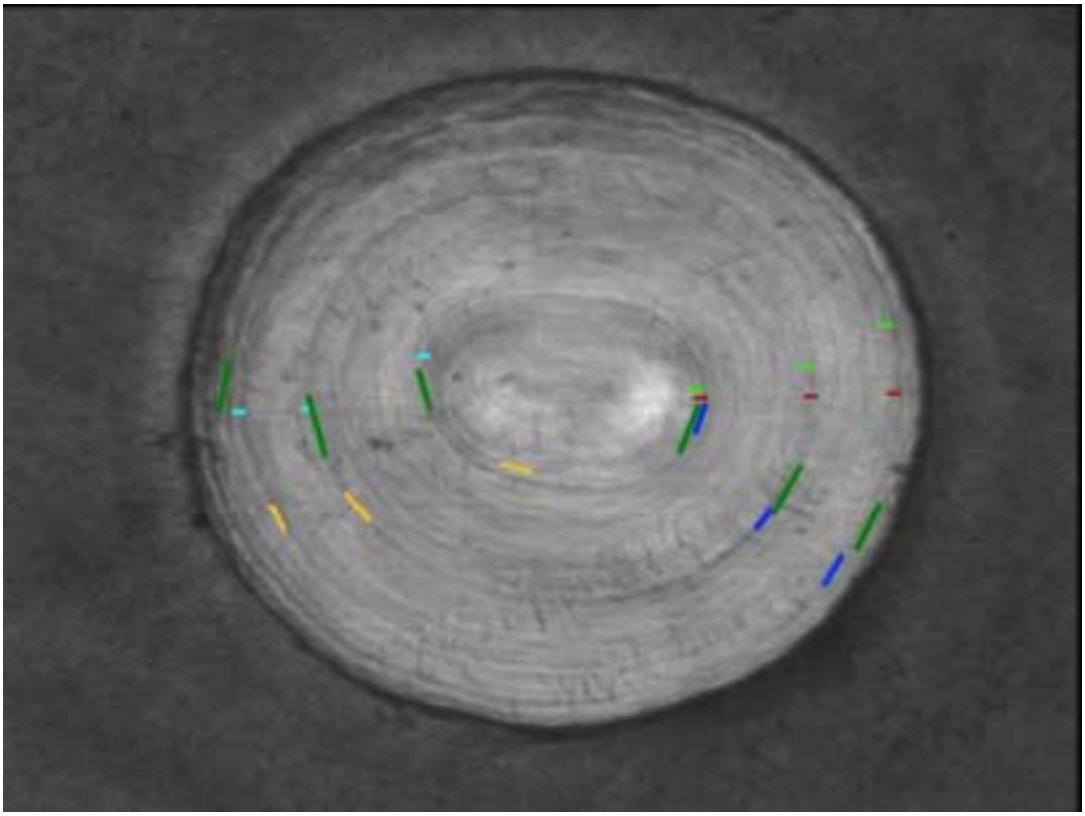

Image 3. *L. piscatorius.* Agreed Age 3. File name: 67-99-5b-3. Collection North. Total Agreement by experienced readers.

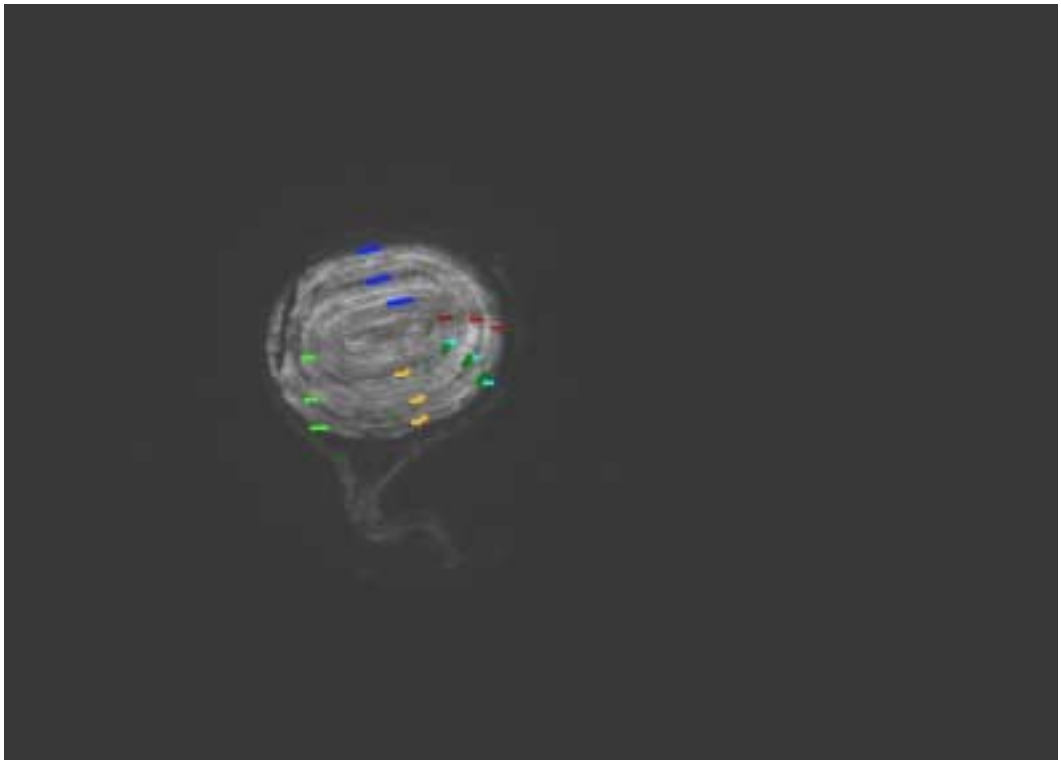

Image 4. *L. piscatorius.* Agreed Age 3. File name: 2-1b-4 2000. Collection South. Total Agreement by experienced readers.

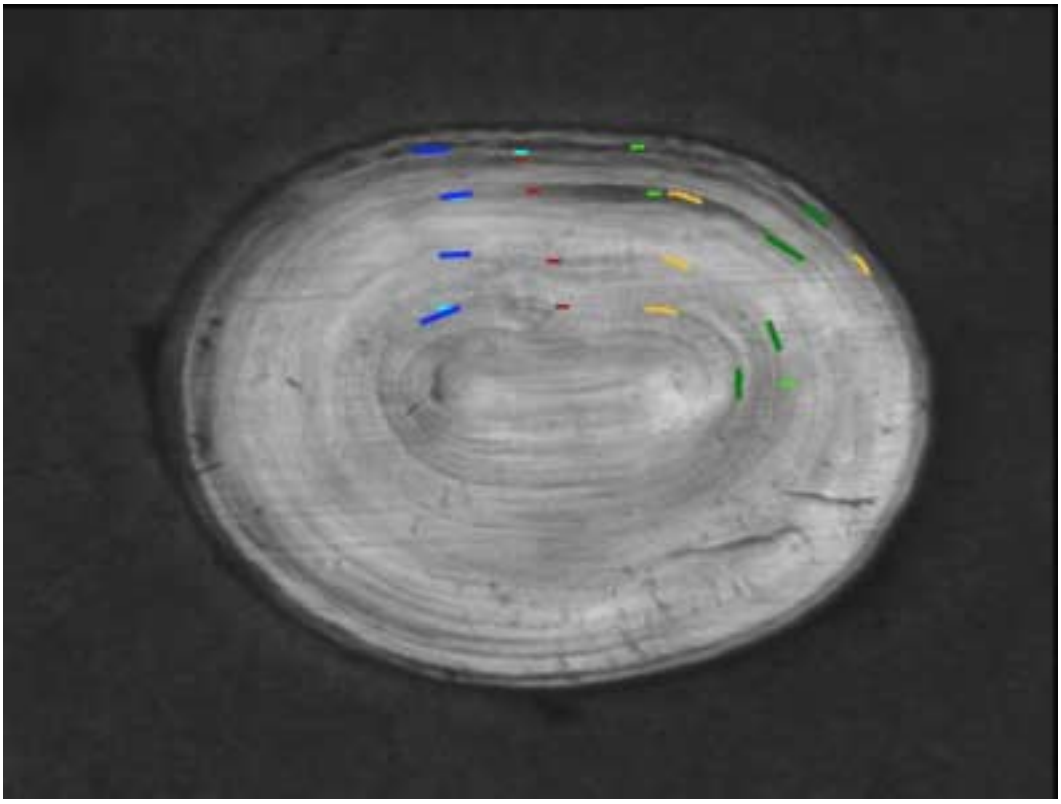

Image 5. *L. piscatorius.* Agreed Age 4. File name: 60-99-3a-3. Collection North. Total Agreement by experienced readers.

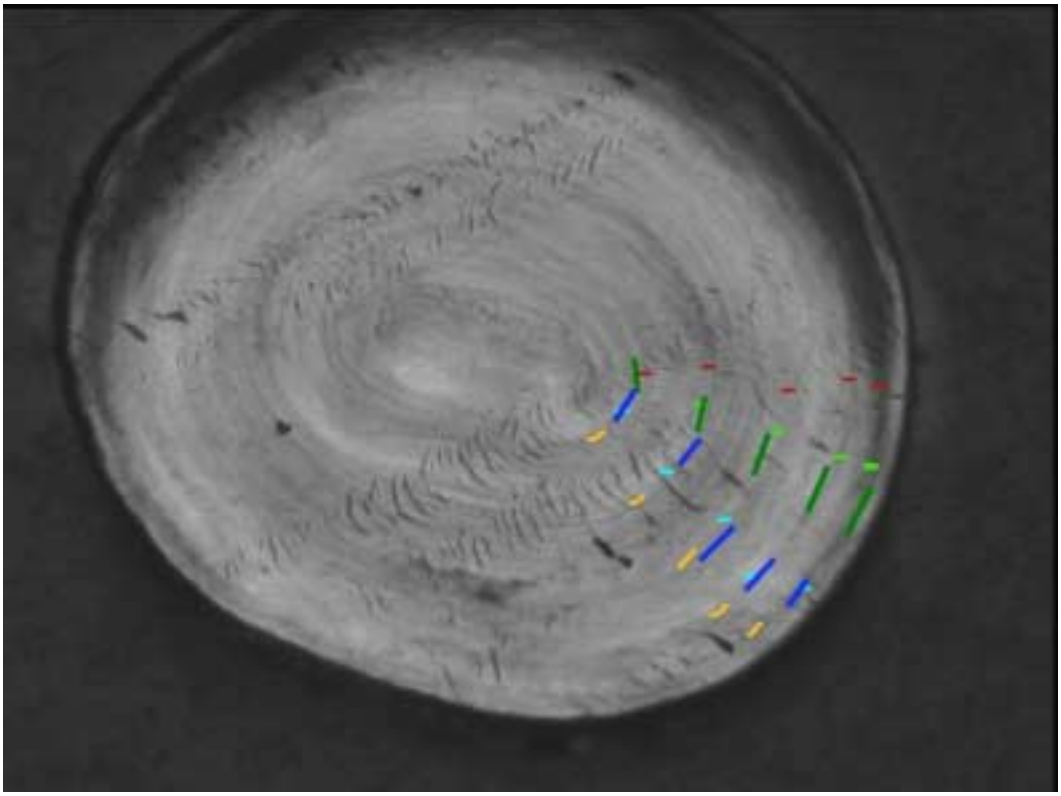

Image 6. *L. piscatorius.* Agreed Age 5. File name: 73-99-1b-4. Collection North. Total Agreement, better image.

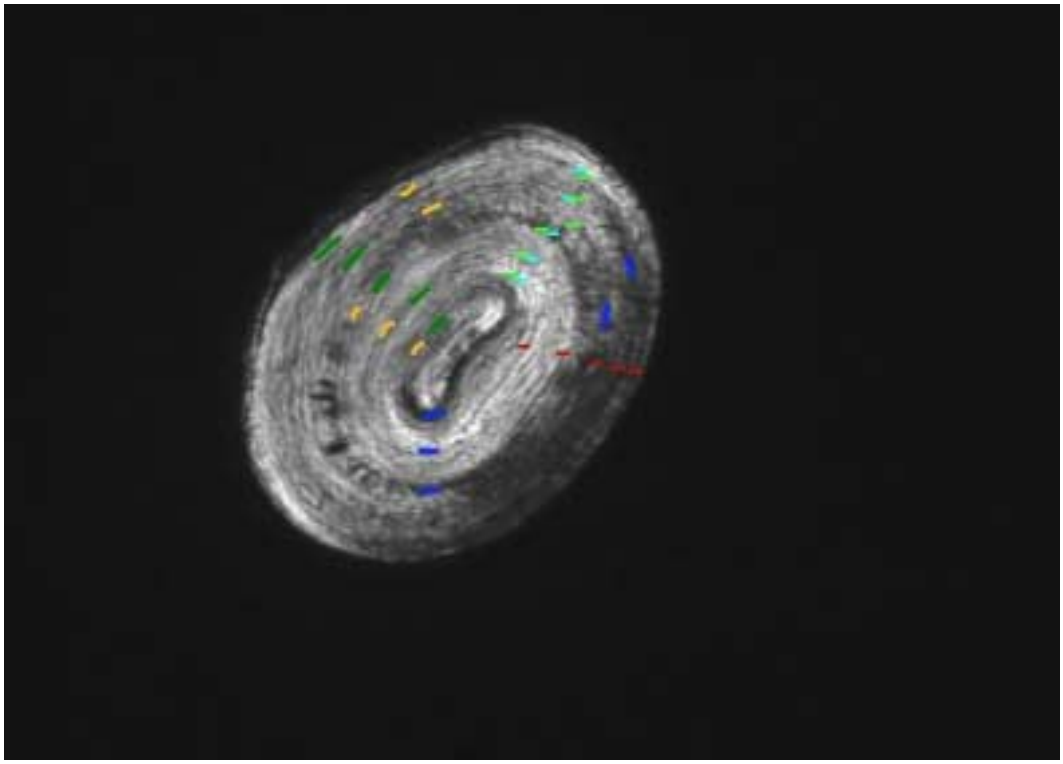

Image 7. *L. piscatorius.* Agreed Age 5. File name: 13-4a-21999. Collection South. Total Agreement by experienced readers.

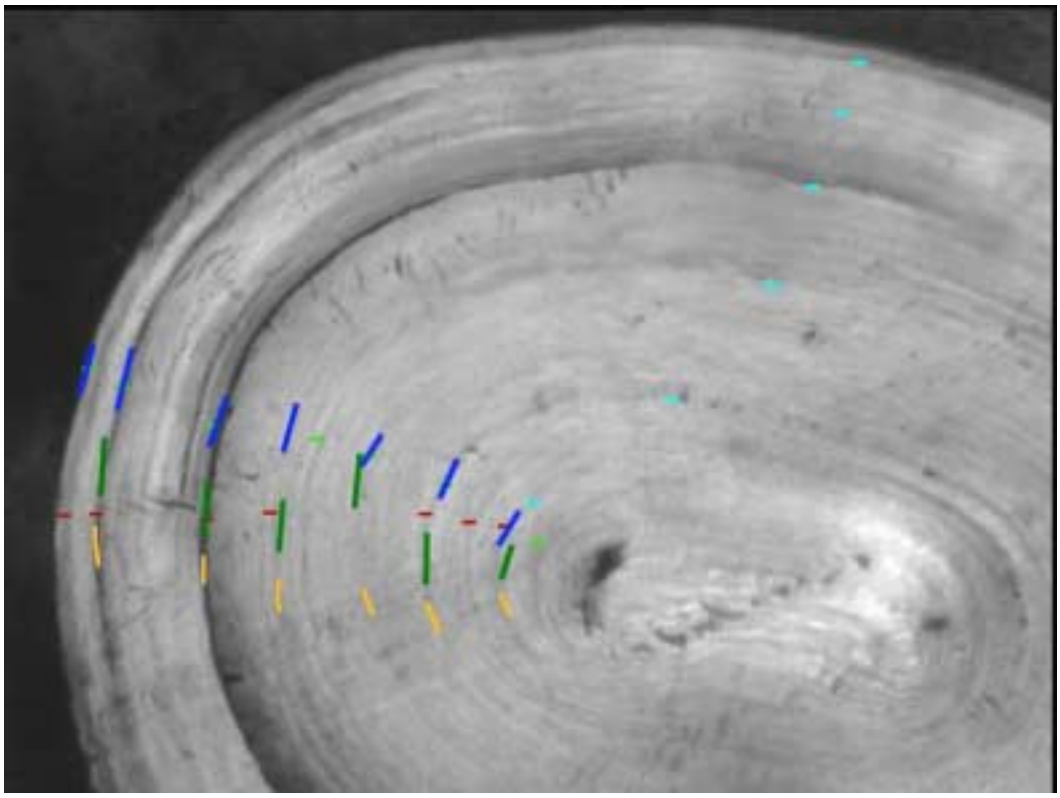

Image 8. *L. piscatorius.* Agreed Age 6. File name: 2b-5. Collection North. Modal Age agreed, but different rings selected.

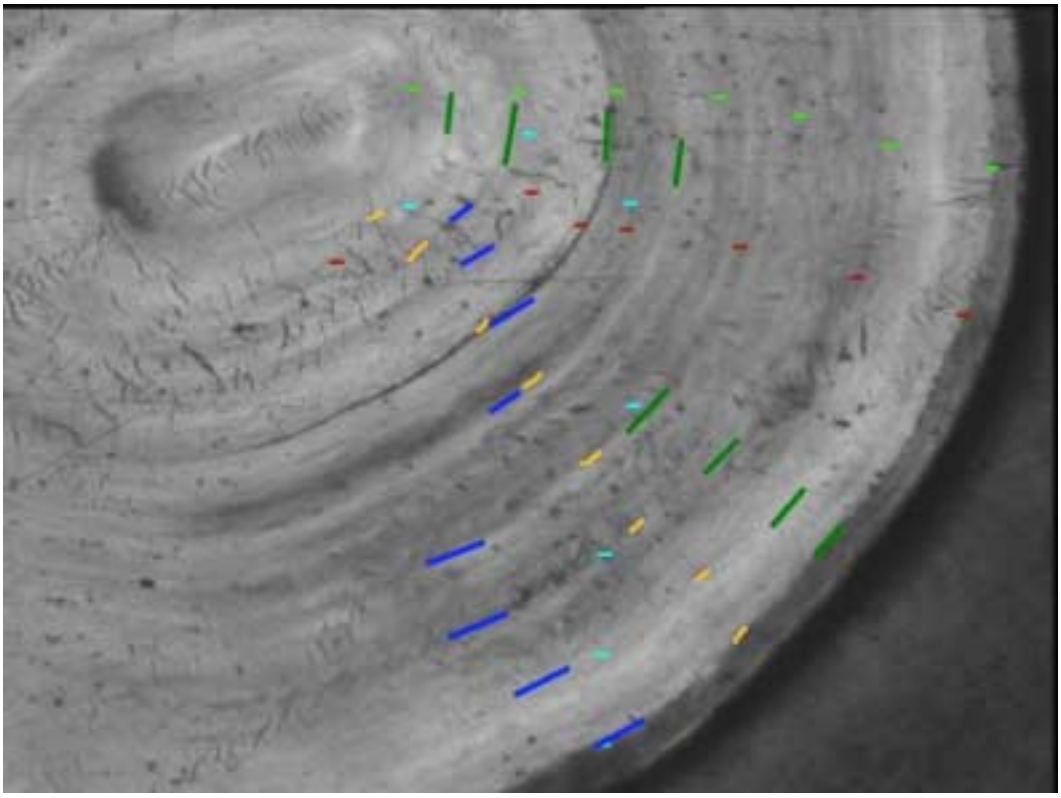

Image 9. *L. piscatorius.* Agreed Age 8. File name: 66-99-4a-7. Collection North. Disagreement on 3rd and 4th rings.

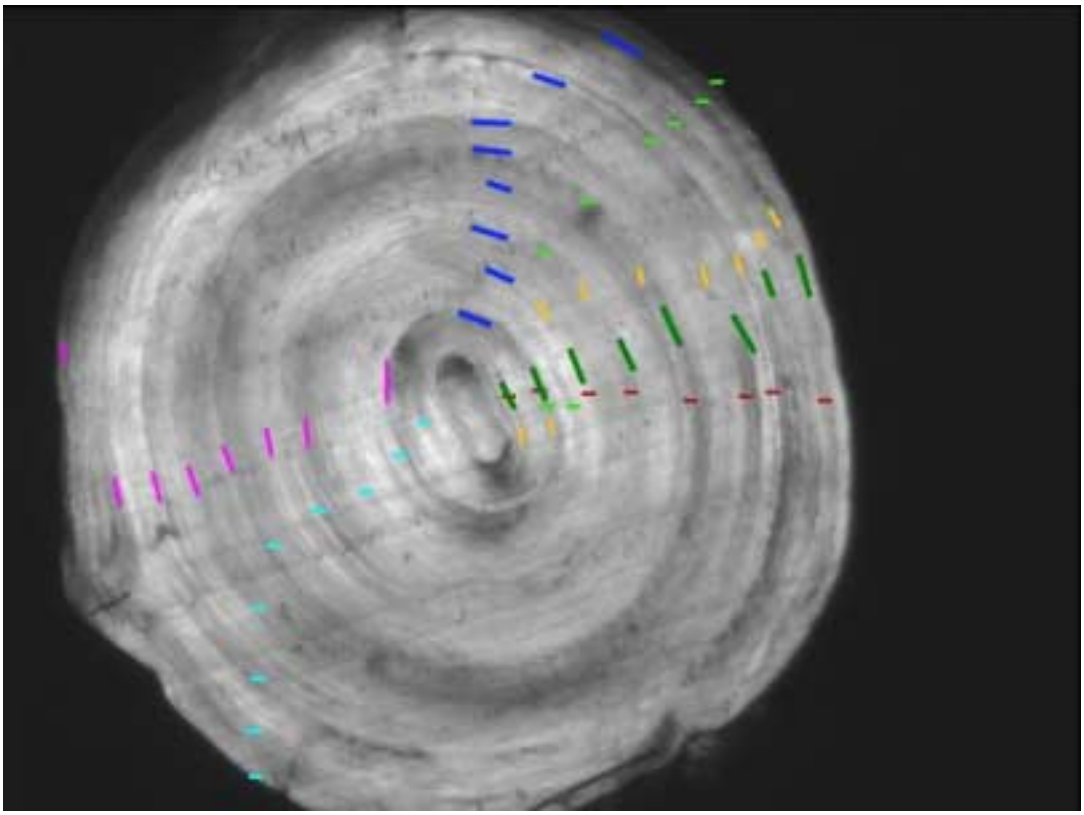

Image 10. *L. piscatorius.* Agreed Age 9. File name: 11-00-1a-1. Collection North. Good agreement by R5 &R6, R3= N-1.

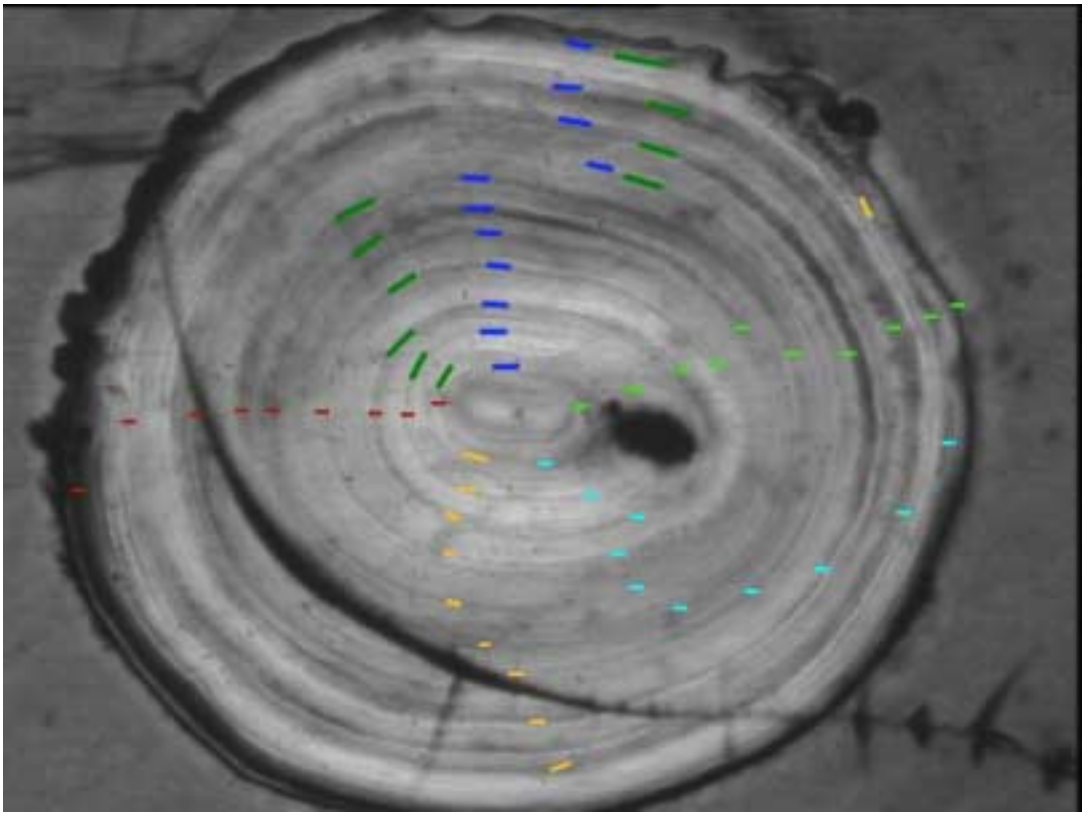

Image 11. *L. piscatorius.* Agreed Age 10. File name: 84-99-2A-3. Collection North. Total Agreement by experienced readers.

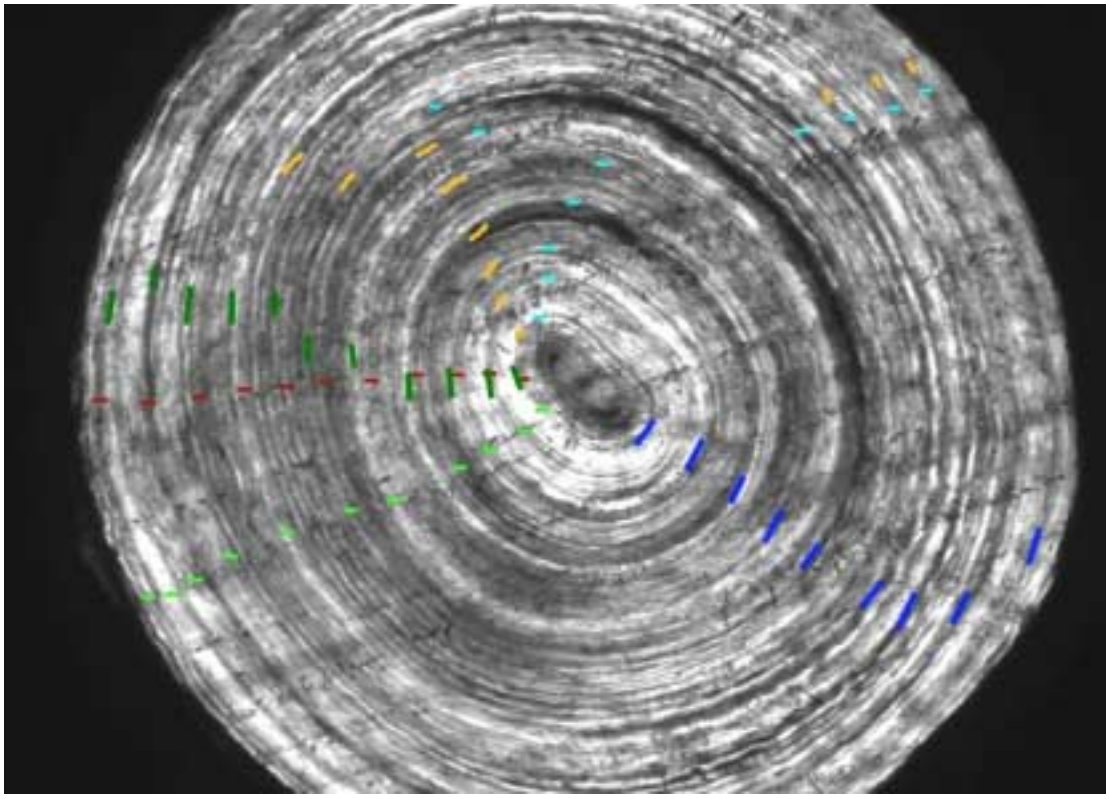

Image 12. *L. piscatorius.* Agreed Age 11. File name: 15-4b-5 1999. Collection South. R5&3=11, R6=12, however chose very similar rings.

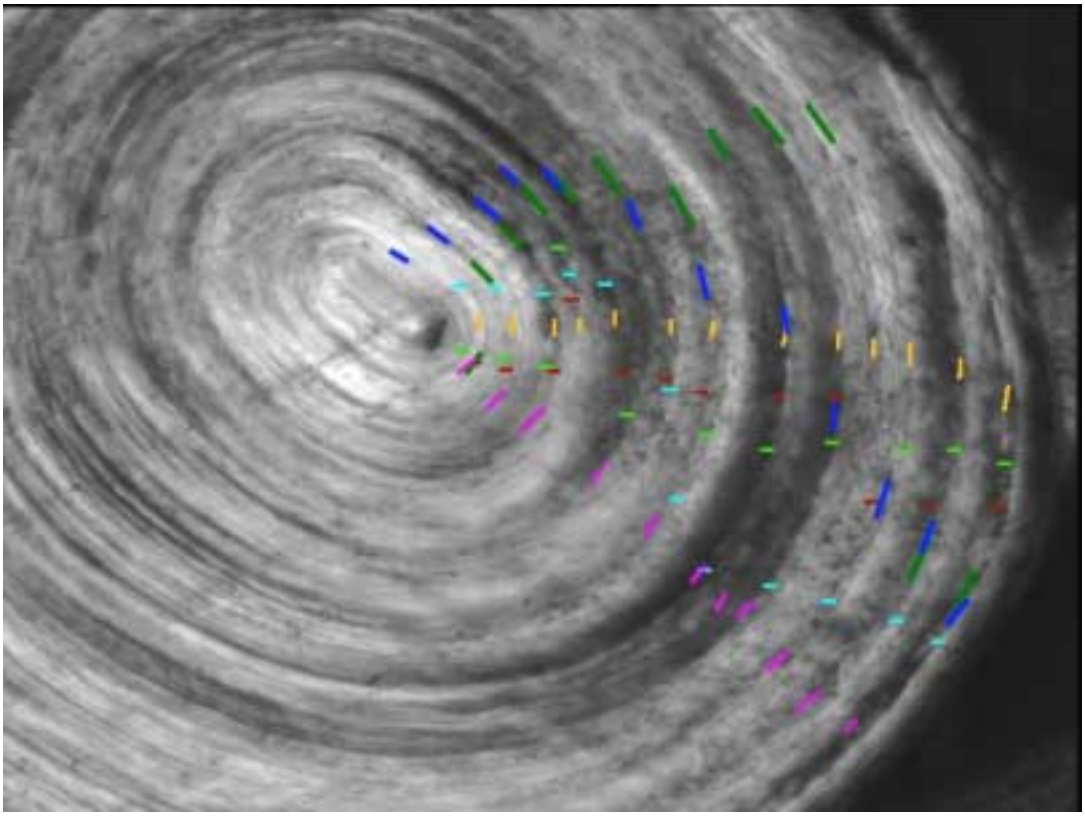

Image 13. *L. piscatorius.* Agreed Age 12. File name: 114-99-2a-1. Collection North. Disagreement on one ring.

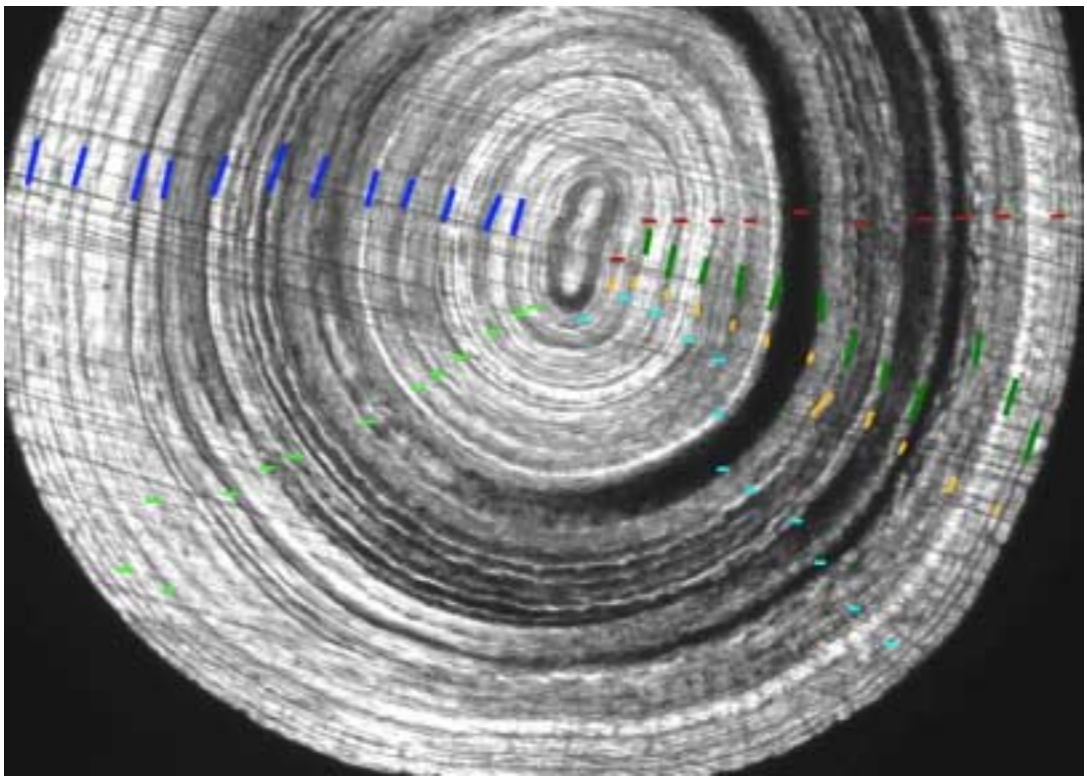

Image 14. *L. piscatorius.* Agreed Age 12. File name: 8-4b-3-2000. Collection South. R3 differs on ring 1 and 11.

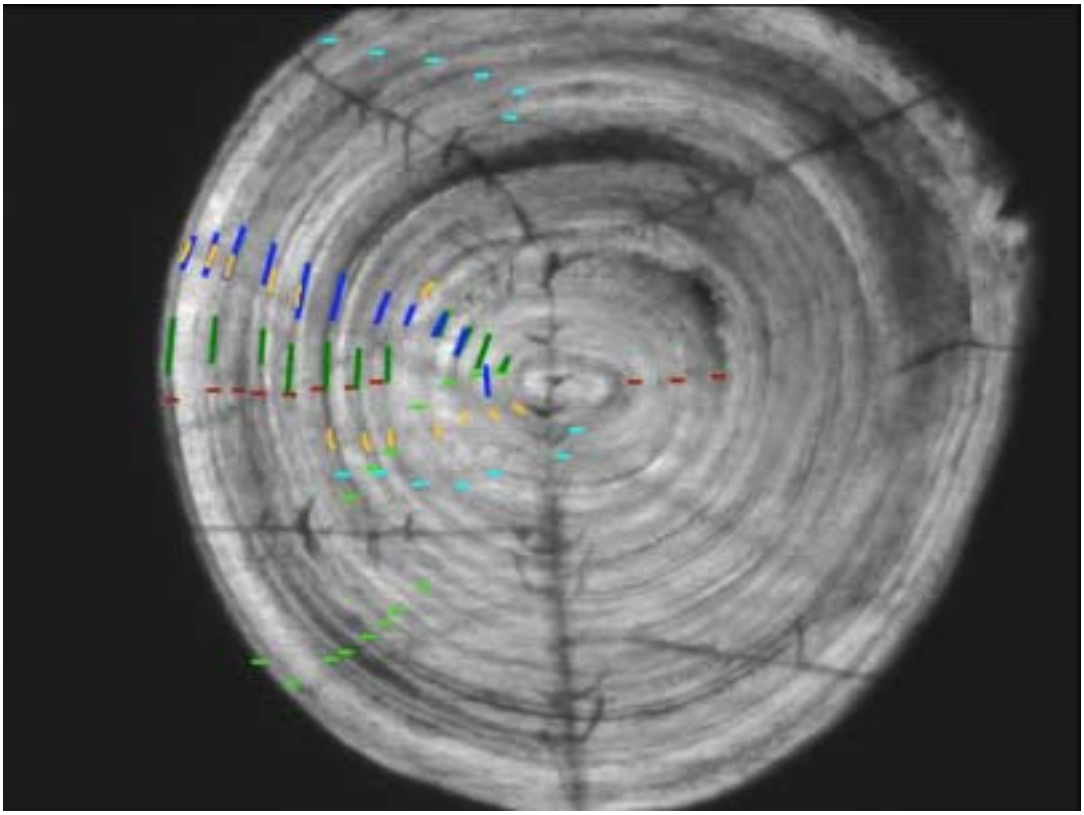

Image 15. *L. piscatorius.* Agreed Age 13. File name: 238-97-1a-4. Collection North. R1,5 &6 =13, similar rings, R3=11.

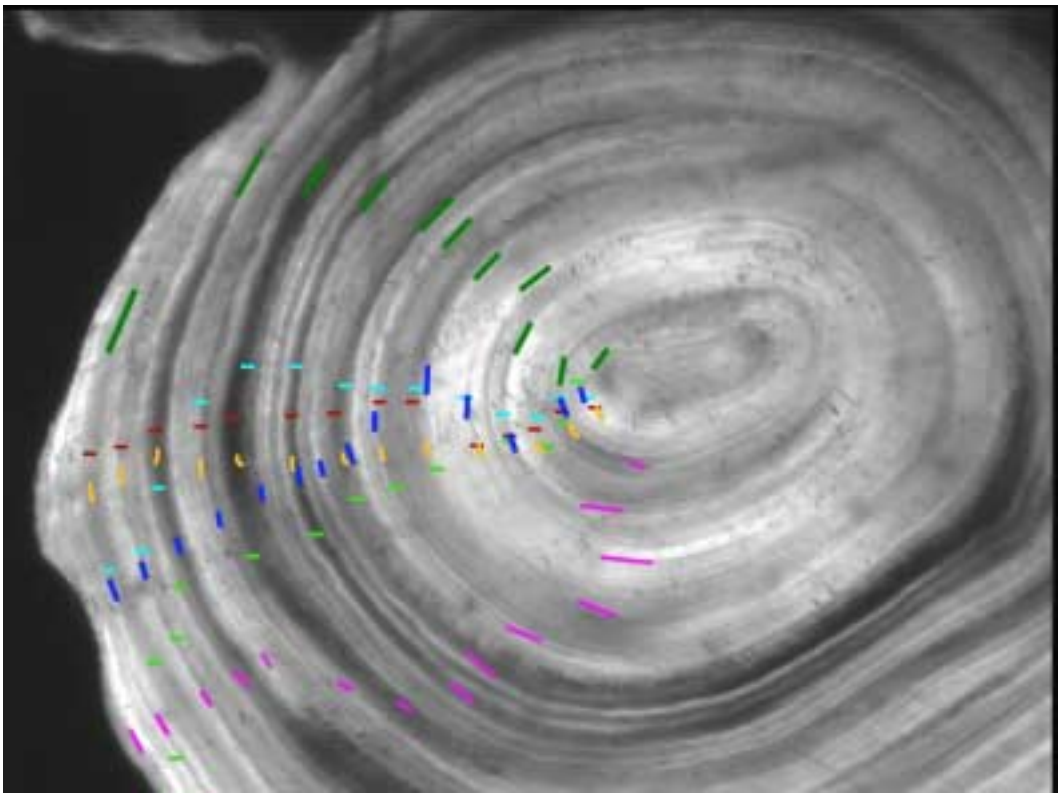

Image 16. *L. piscatorius.* Agreed Age 14. File name: 100-99-3b-3. Collection North. R5&6 in total agreement, R3=11.

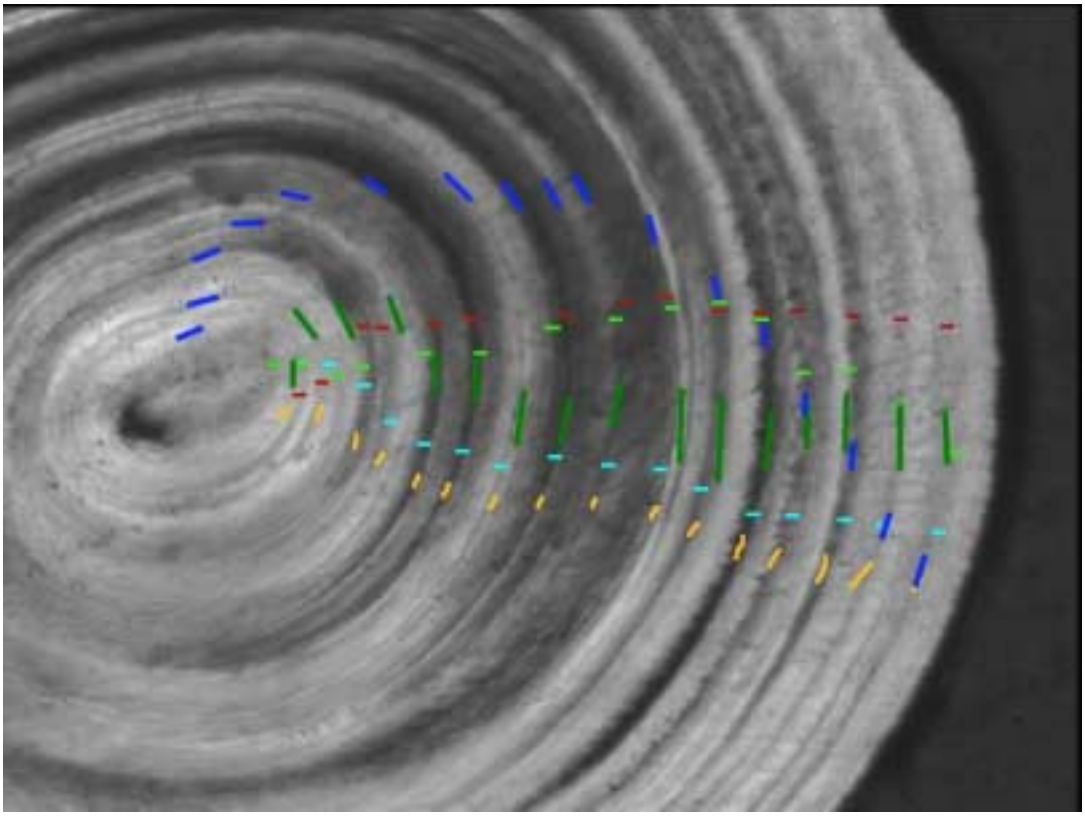

Image 17. *L. piscatorius.* Agreed Age 16. File name: 77-99-5b-8. Collection North. Total Agreement by experienced readers.

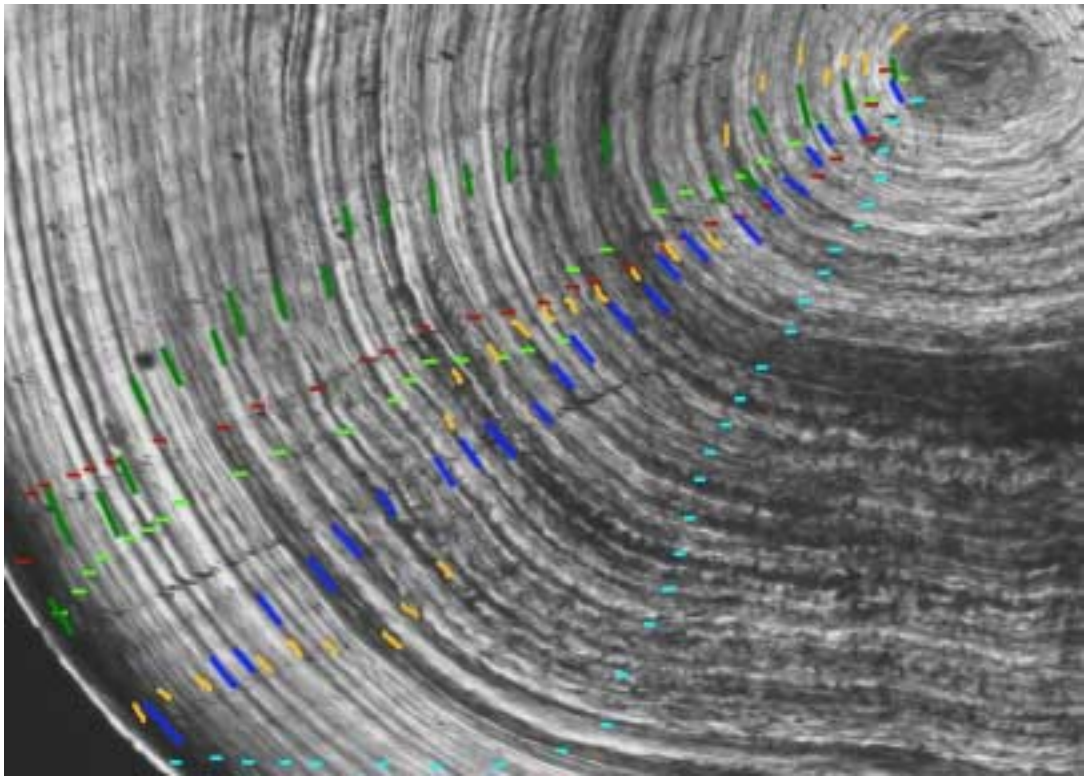

Image 18. *L. piscatorius.* Agreed Age 24. File name: 9-1b-3 1999. Collection South. Age 24-29, image looks good, different rings chosen.

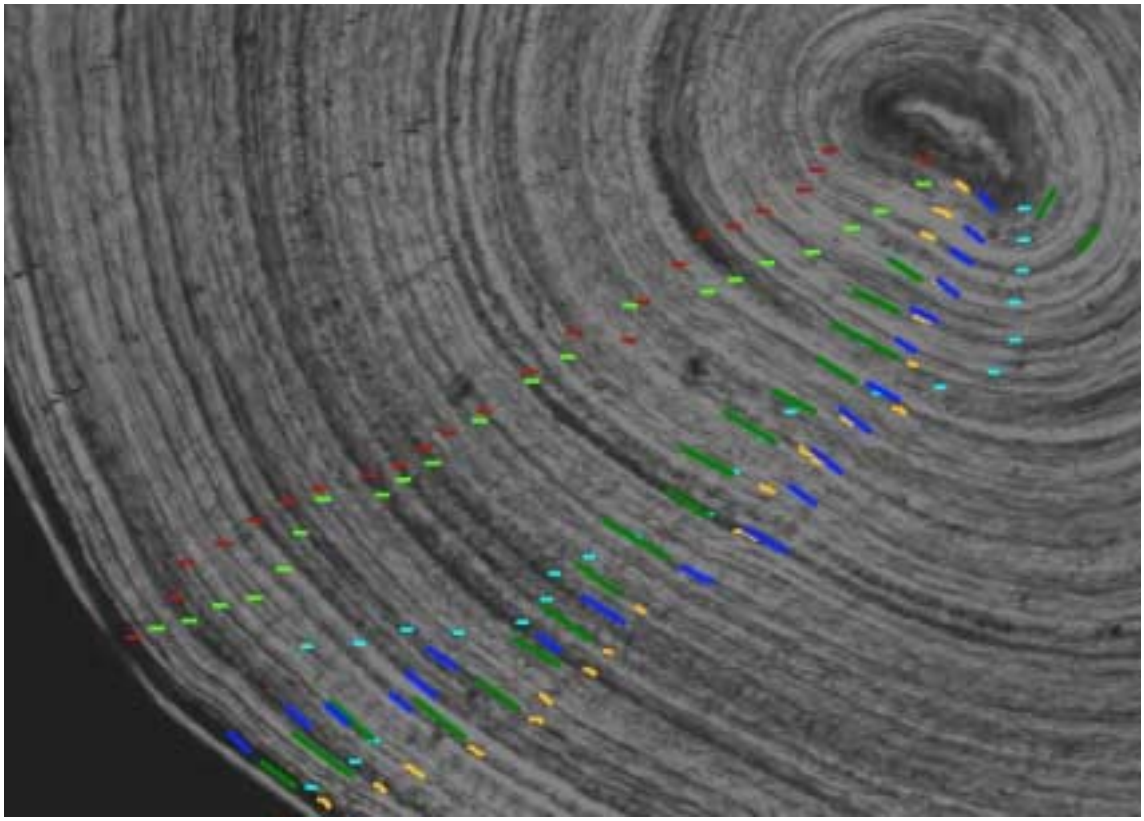

Image 19. *L. piscatorius.* Agreed Age 25. File name: 9-1b-2 1999. Collection South. Total Agreement by experienced readers.

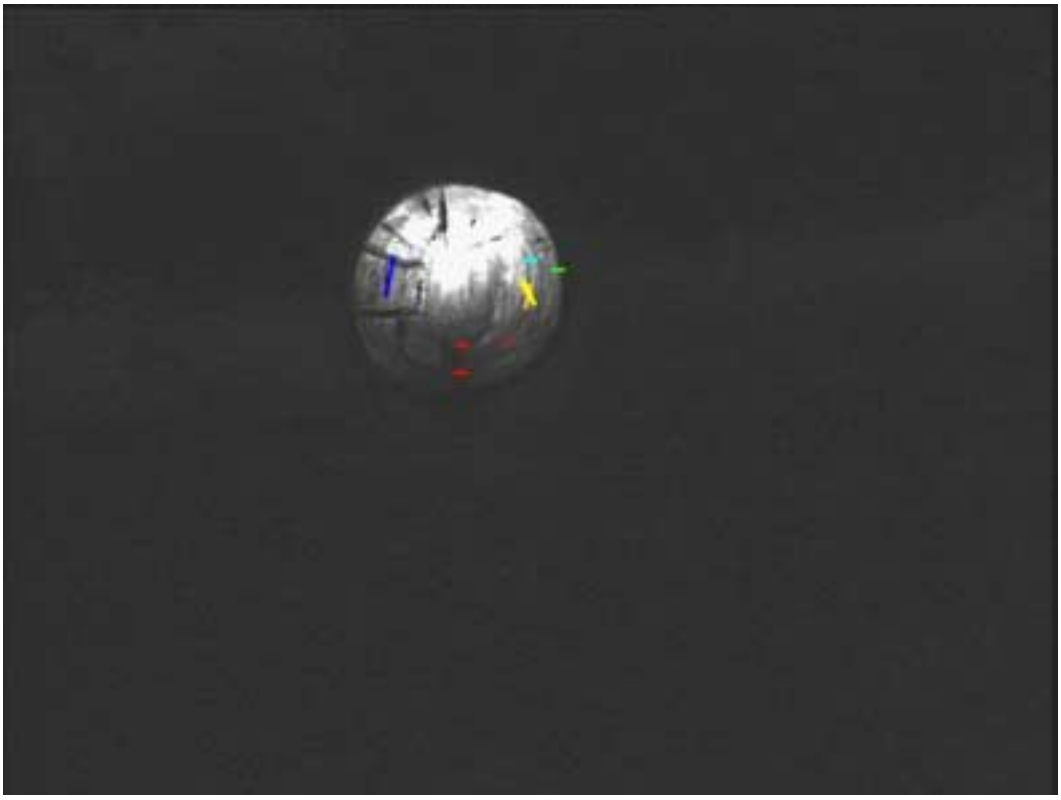

Image 20. *L. budegassa.* Agreed Age 1. File name: A-2. Collection South. Total Agreement by experienced readers.

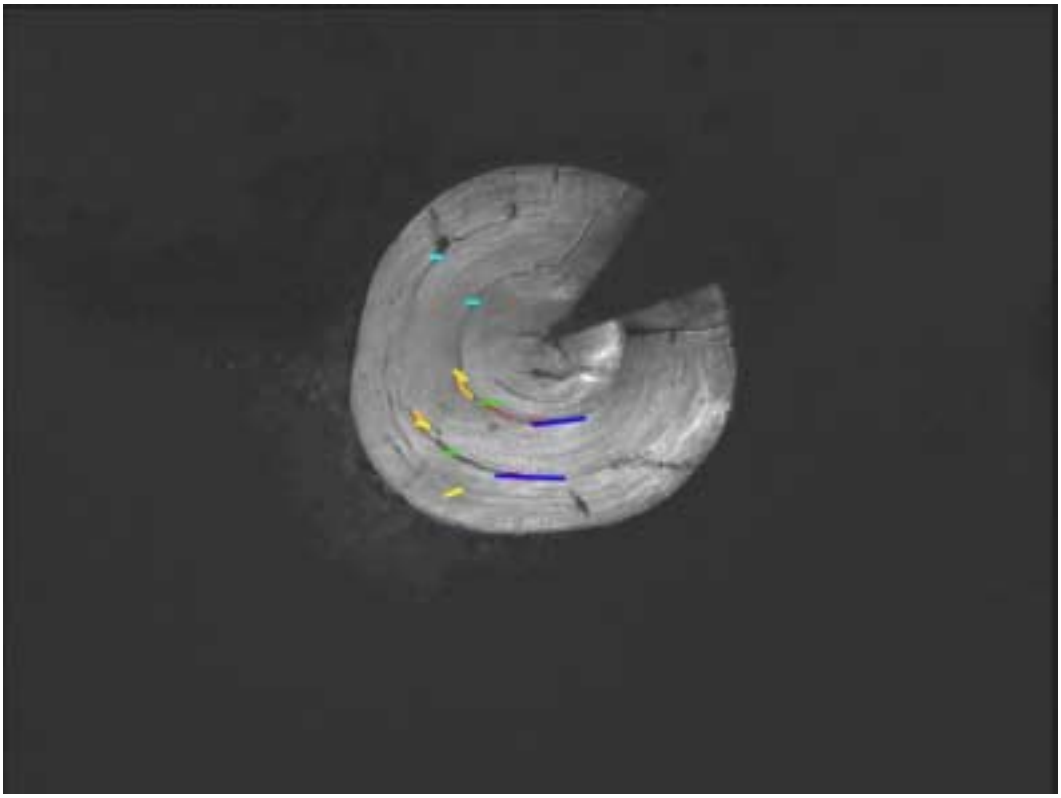

Image 21. *L. budegassa.* Agreed Age 2. File name: A-7. Collection South. Total Agreement by experienced readers.

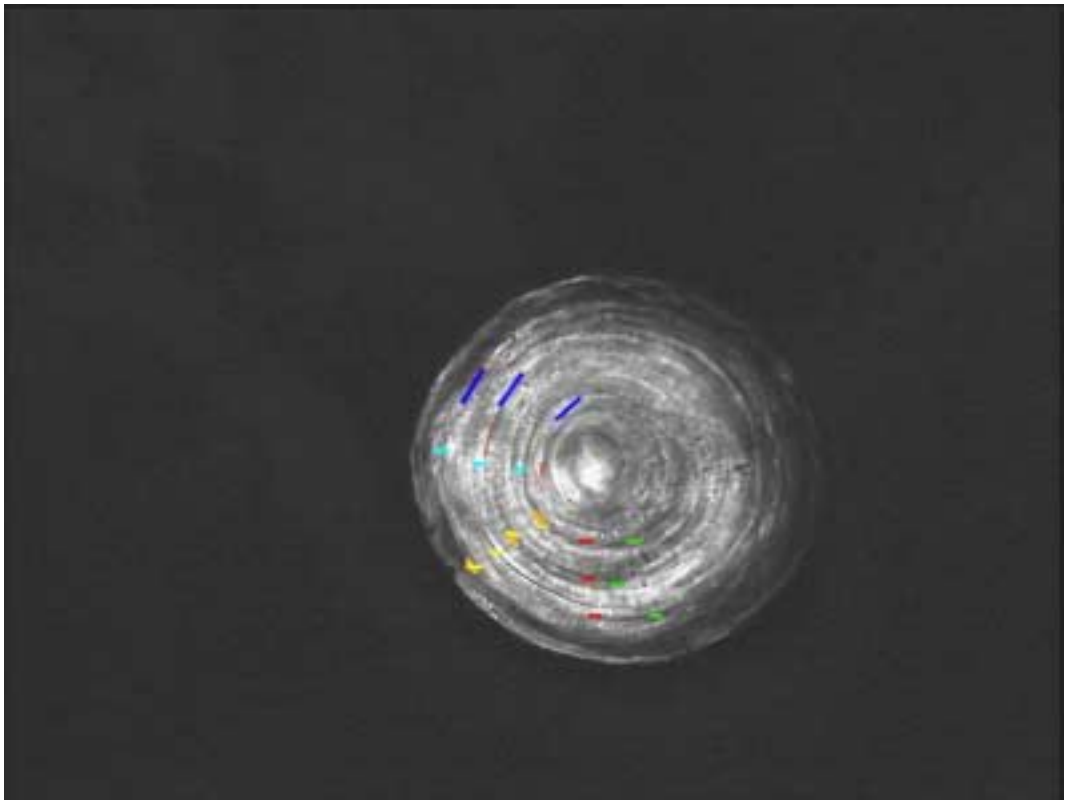

Image 22. *L. budegassa.* Agreed Age 3. File name: B-3. Collection South. Total Agreement by experienced readers.

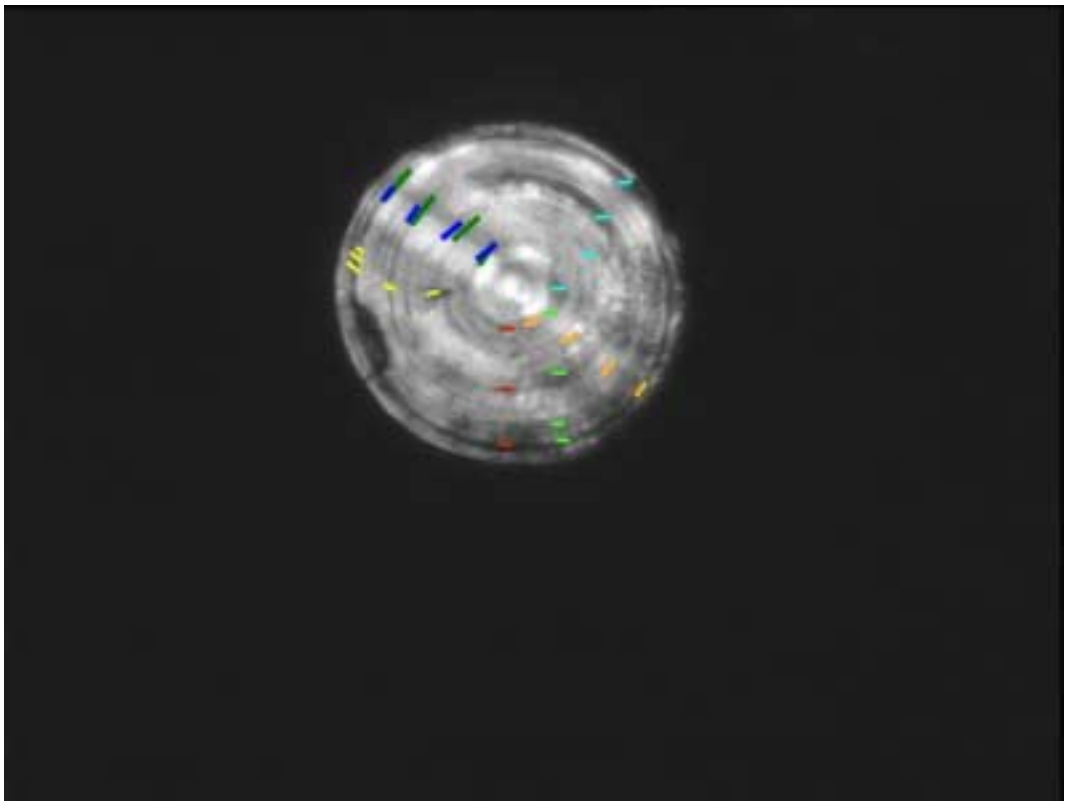

Image 23. *L. budegassa.* Agreed Age 4. File name: 19-1999-5b-8. Collection North. Total Agreement by experienced readers.

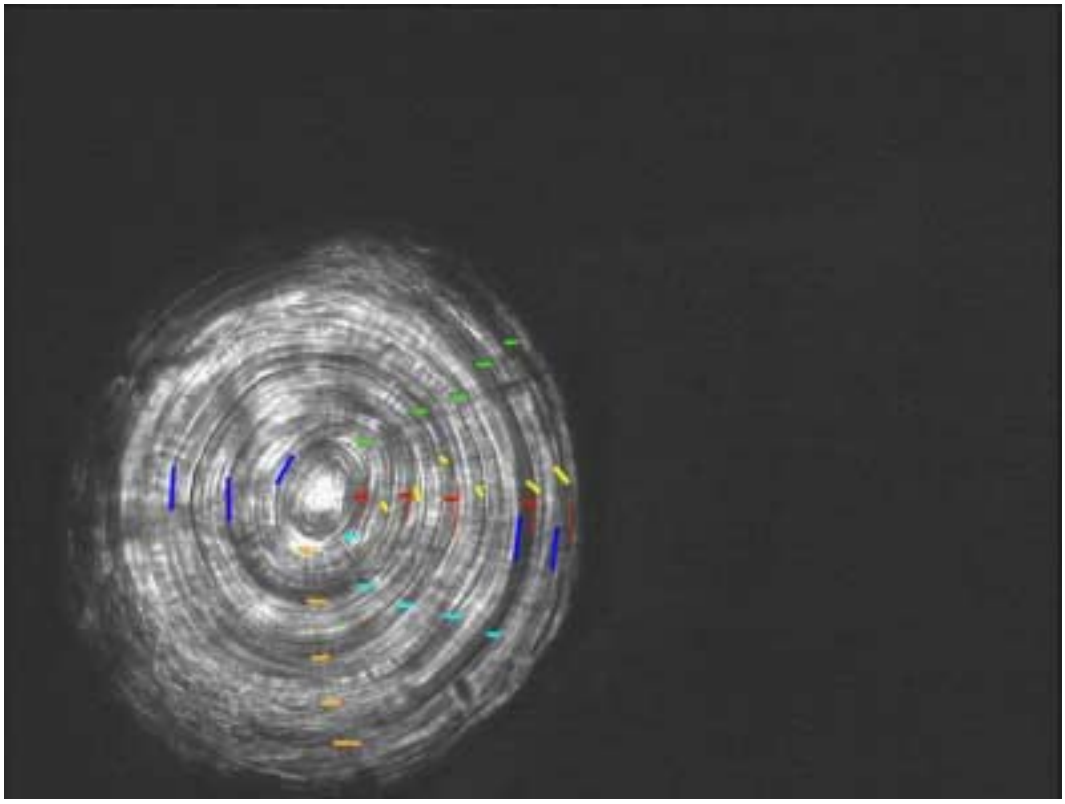

Image 24. *L. budegassa.* Agreed Age 5. File name: C-7. Collection South. Total Agreement by experienced readers.

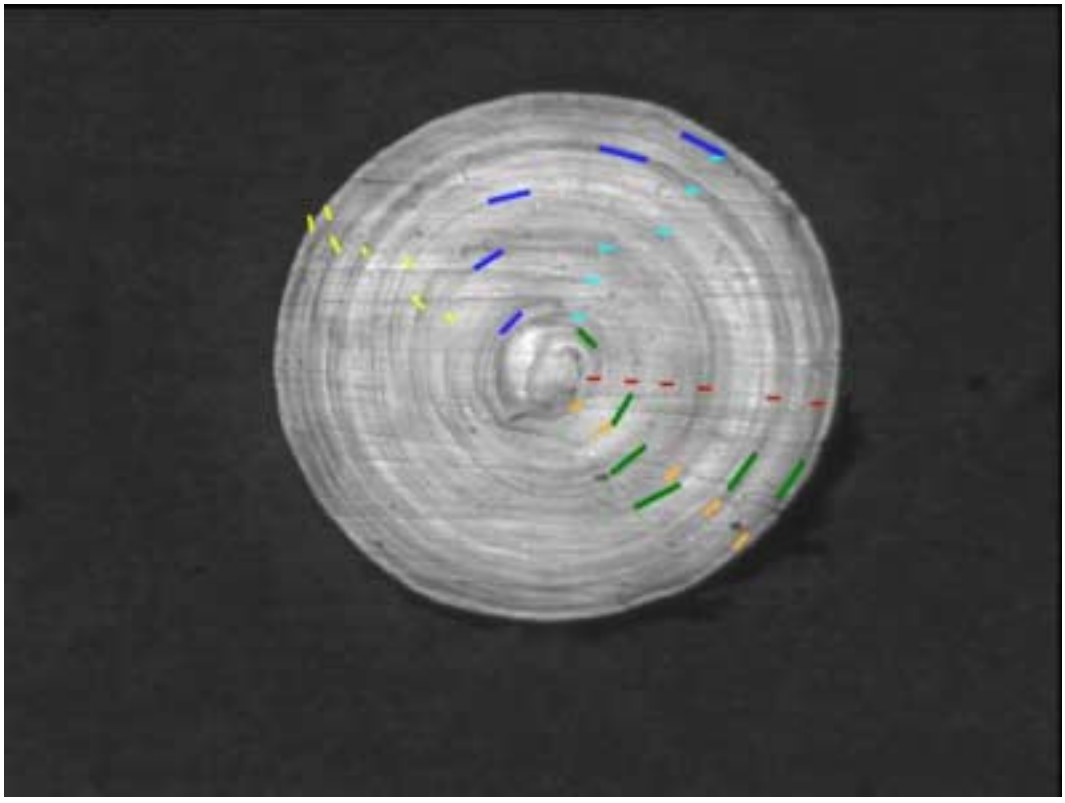

Image 25. *L. budegassa*. Agreed Age 6. File name: 3-1999-3b-2. Collection North. Total Agreement by experienced readers.

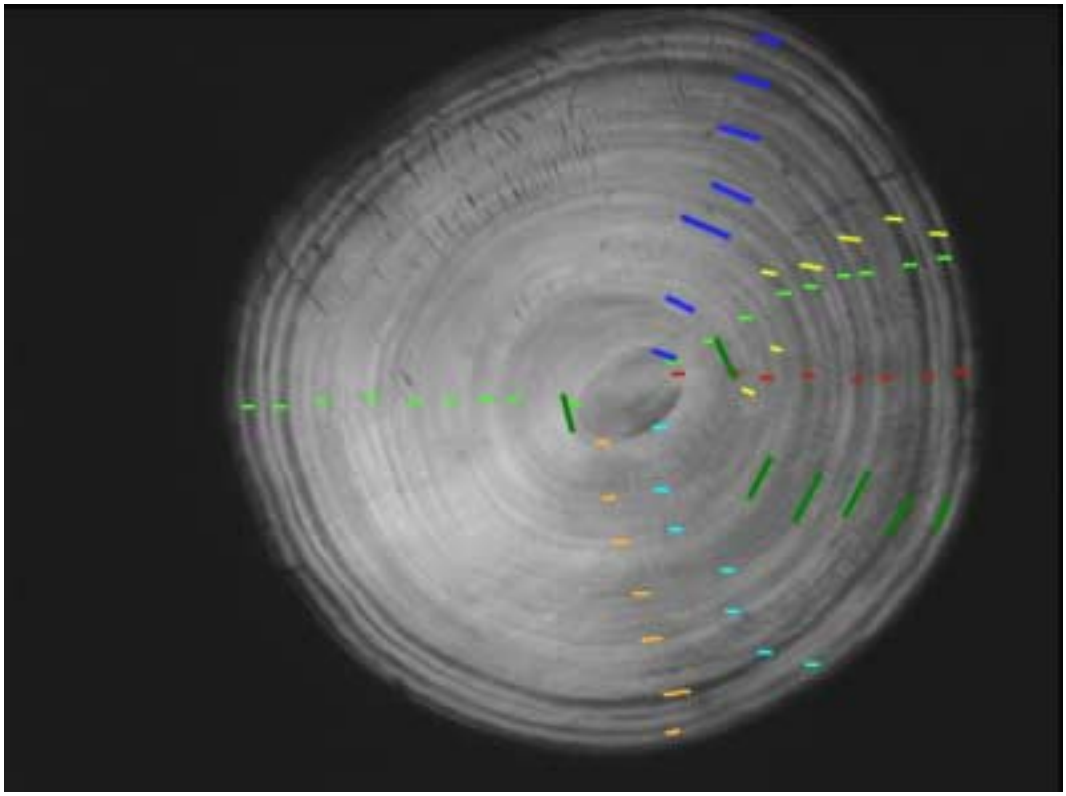

Image 26. *L. budegassa.* Agreed Age 7. File name: 39-1998-3a-3. Collection North. Total Agreement by experienced readers.

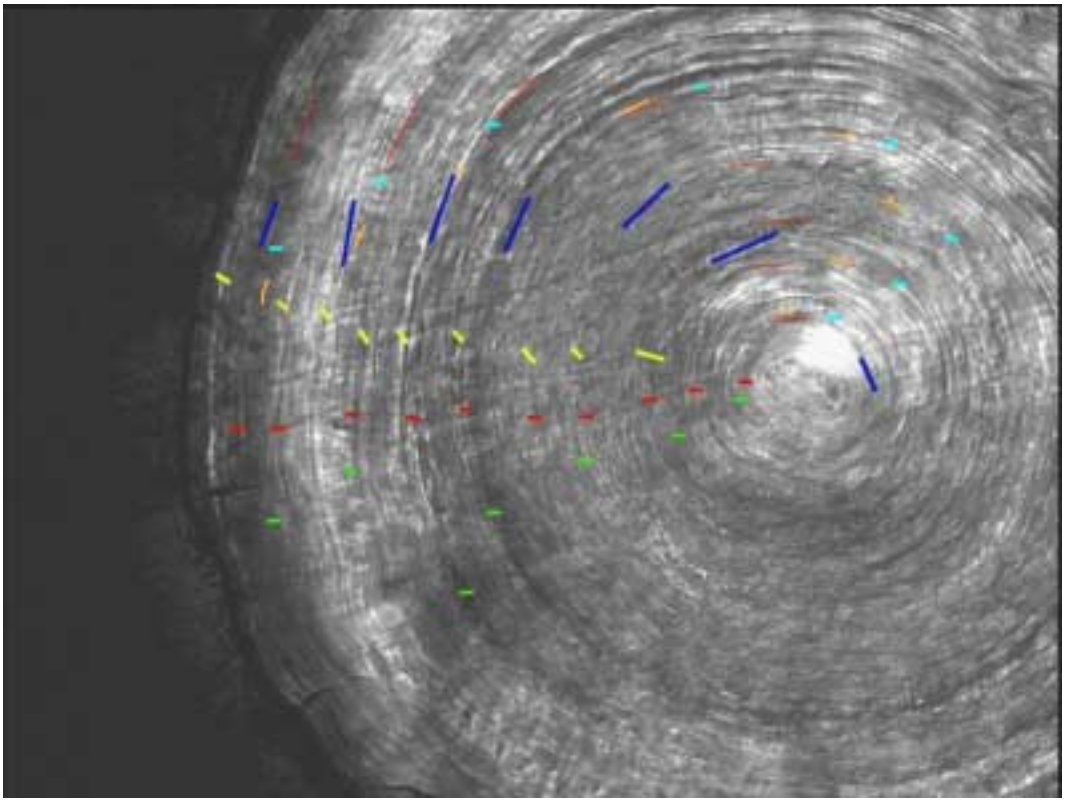

Image 27. *L. budegassa.* Agreed Age 8. File name: G-7. Collection South. R3 missed 3rd ring.

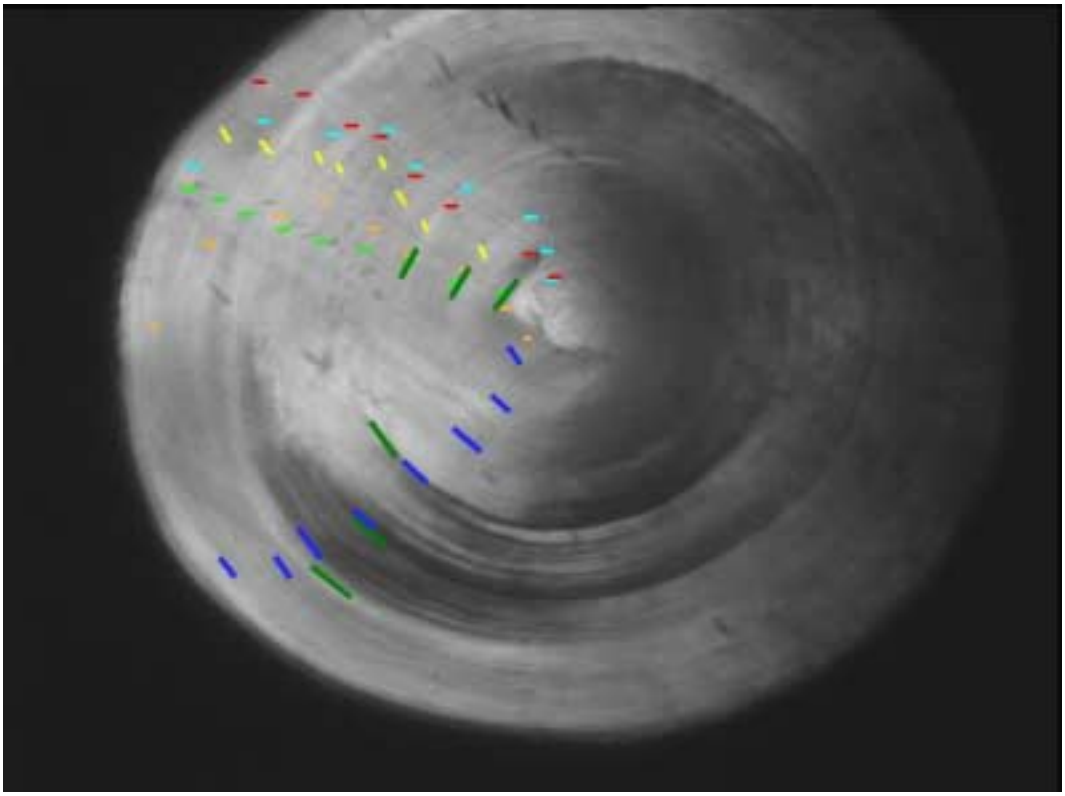

Image 28. *L. budegassa.* Agreed Age 9. File name: 6-1999-3b-4. Collection North. R5&R6=9, similar rings, R3=6.

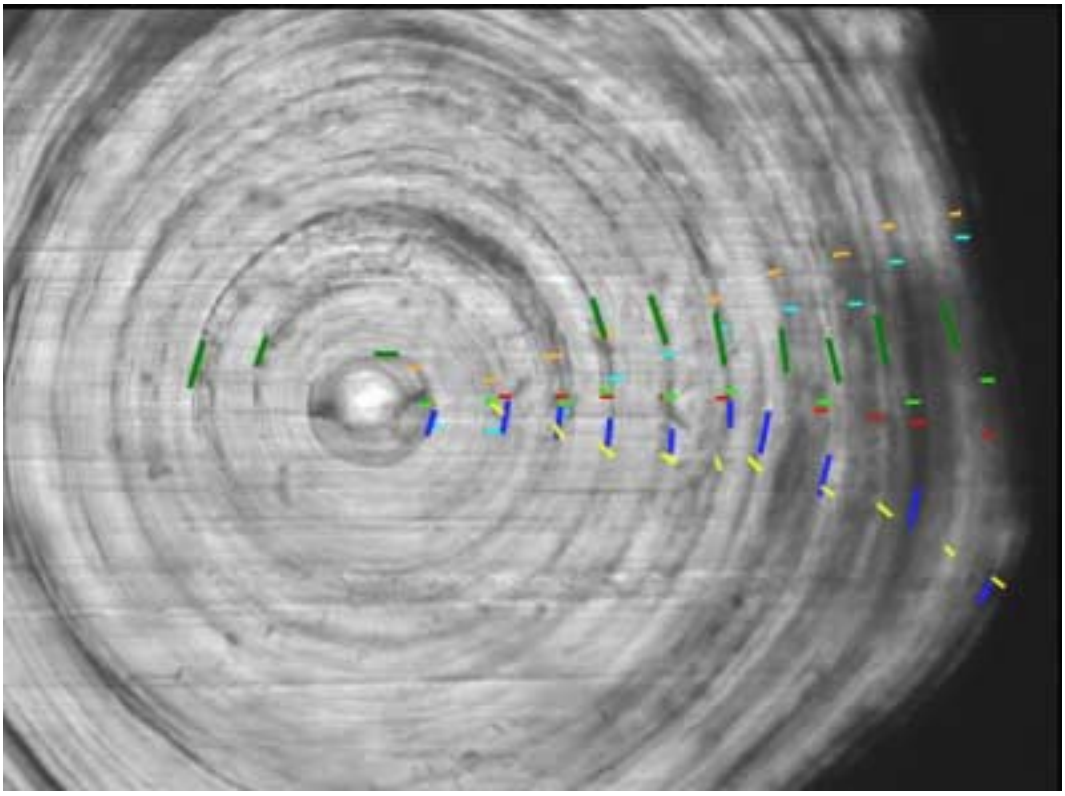

Image 29. *L. budegassa*. Agreed Age 10. File name: 237-1997-5a-5. Collection North. Total Agreement by experienced readers.

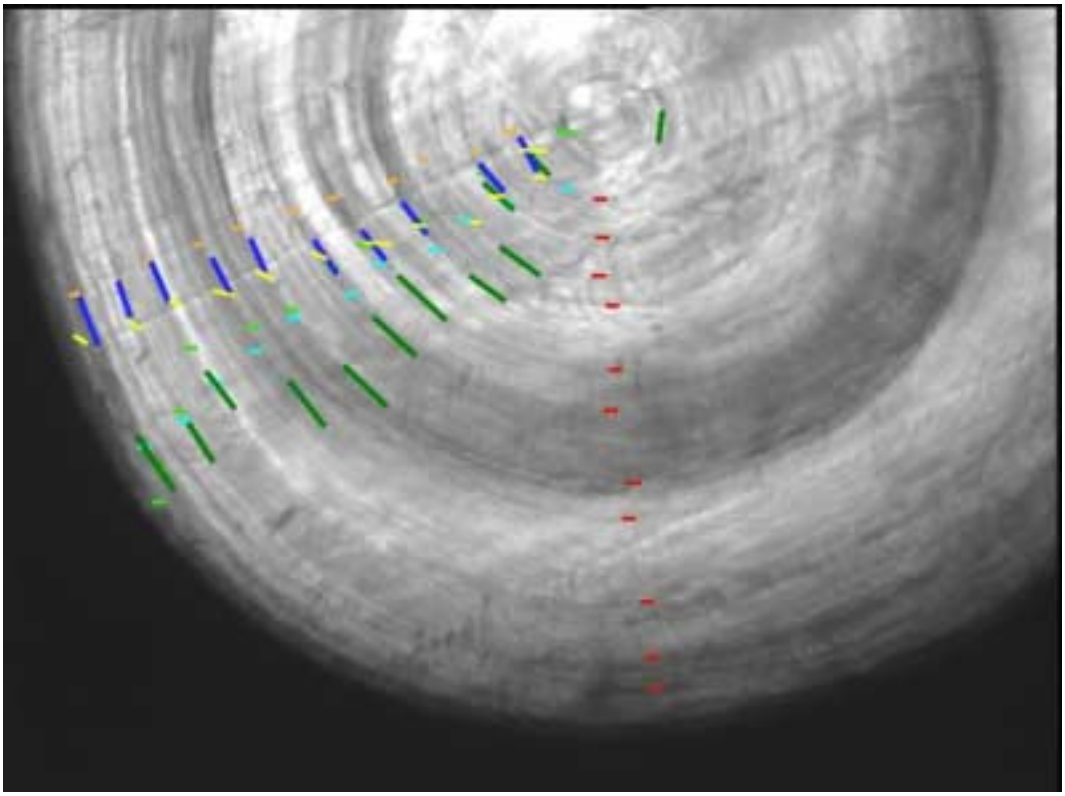

Image 30. *L. budegassa.* Agreed Age 11. File name: 92-1999-5b-10. Collection North. Agreement on all rings except 1st by R3.

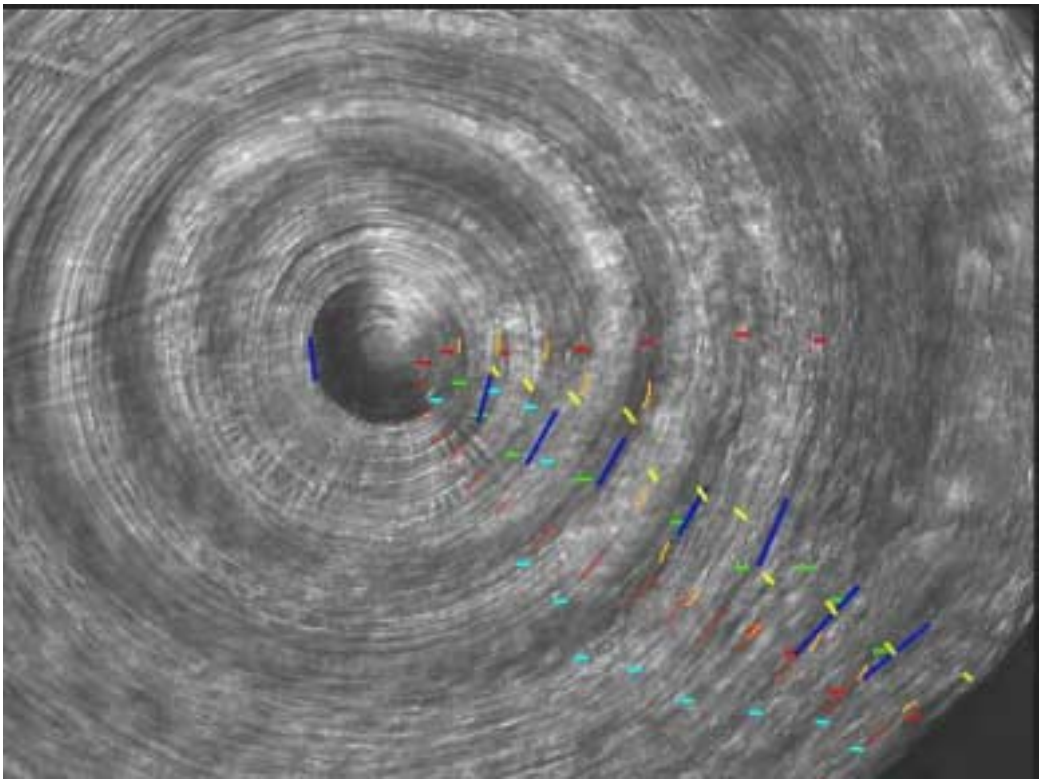

Image 31. *L. budegassa.* Agreed Age 12. File name: K-6 . Collection South. R5&6 = 12, R3=8, Good illustration of applying different ageing criteria.

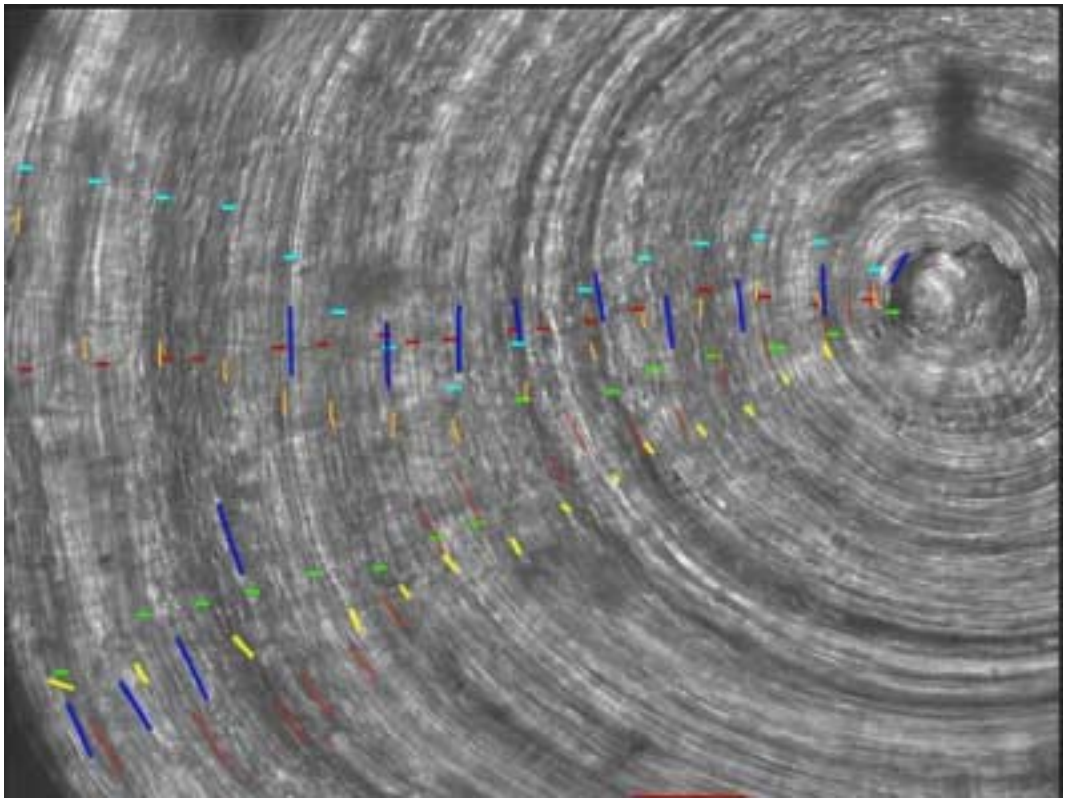

Image 32. *L. budegassa.* Agreed Age 15. File name: N-1. Collection South. R5&6=15, R3=13.

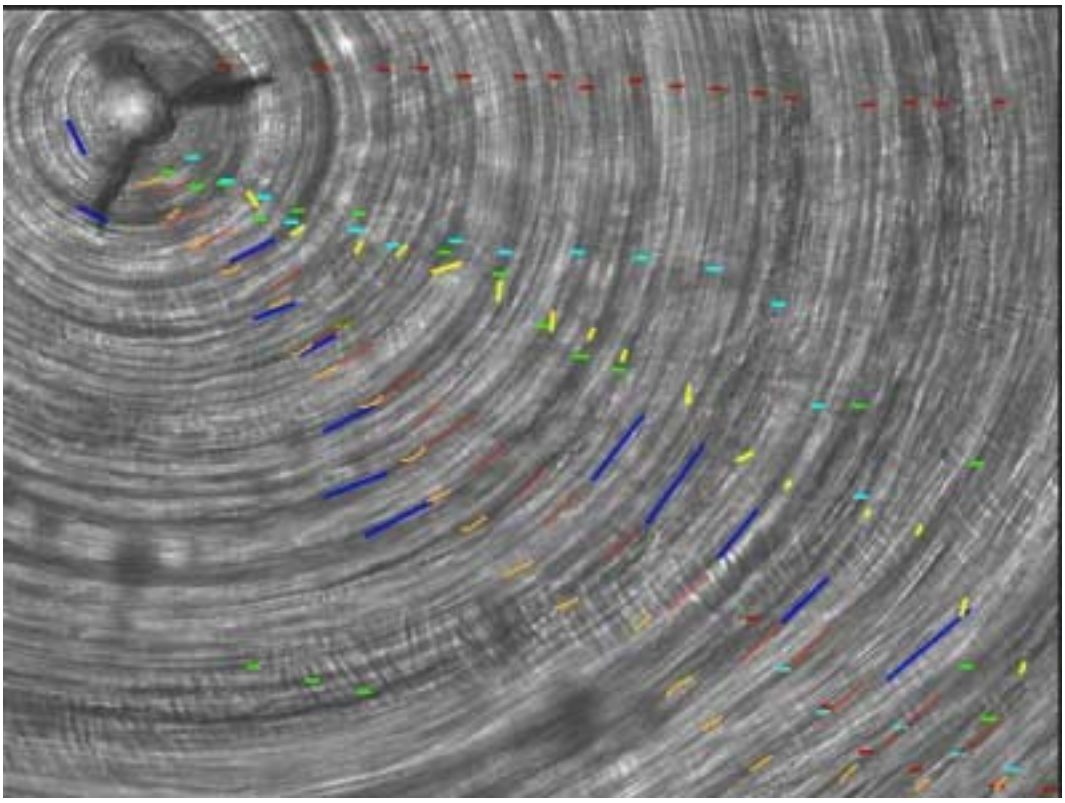

Image 33. *L. budegassa*. Agreed Age 19. File name: N-5. Collection South. R3=17, R6=19 and R5=20, R5&6 chose similar rings.

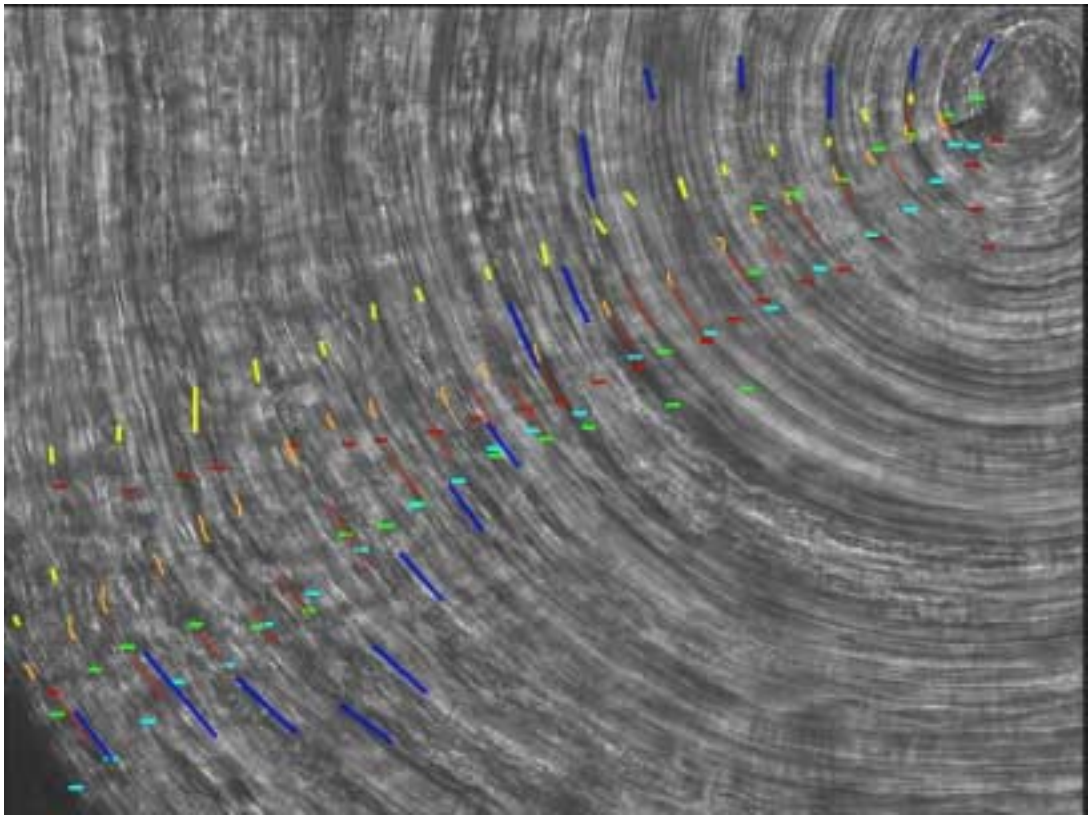

Image 34. *L. budegassa.* Agreed Age 21. File name: O-3. Collection South. R3=16, R5&R6=21, but chose some different rings.